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# BLOOD VOLUME IN WOUNDED SOLDIERS.

## I. Blood Volume and Related Blood Changes after Hemorrhage.

By OSWALD H. ROBERTSON, M.D.,
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(Received for publication, October 3, 1918.)

### INTRODUCTION.

Our knowledge on the subject of hemorrhage and the subsequent restoration of the circulation is in a state of rapid change. New facts are constantly coming to light which necessitate revision of previous conceptions. Furthermore, the data at hand, although much increased of late, are by no means complete. This work is therefore presented with the realization that the interpretation of some of our findings may be open to question, and that as regards their significance further observations may lead to somewhat different conclusions. However, certain phenomena have occurred so consistently that it has seemed safe to make fairly definite deductions in these instances.

There is a lack of general agreement among investigators on problems of blood loss as to the data which are of most value in determining the actual condition of the patient after hemorrhage, and the ensuing changes which occur in his blood. The principal reason for this diversity of opinion appears to lie in the fact that all the methods heretofore employed in the estimation of blood changes have failed to take into account variations occurring in the volume of the blood. It is well recognized that following hemorrhage the body promptly begins to add fluid to the circulation in an attempt to make up the total blood bulk to its previous amount, but little actual knowledge

exists concerning the rapidity with which this diluting process occurs and the factors controlling it. Thus it is not surprising that the data given by red cell counts, hemoglobin per cent, hematocrit determinations, etc., when taken alone, are often difficult to interpret, since they indicate only the variations occurring in one unit of blood. These same data, however, when viewed side by side with blood volume determinations, take on an entirely different significance. Changes in hemoglobin and red cells, whose cause has heretofore seemed most obscure, become, in the light of this more complete knowledge, readily explainable and are seen to occupy their proper place in relation to a changing blood volume. For example, a marked drop in hemoglobin per cent following the rise produced by transfusion, has been generally regarded as an unfortunate occurrence due to hemolysis or perhaps to some less rapid destruction of the introduced cells. Repeated blood volume estimations have revealed the true nature of this phenomenon, showing that in such cases the volume was under normal, even after the transfusion, and that the drop in hemoglobin was due to progressive dilution of the blood as the volume was made up.

The present study includes a series of twenty-one cases of hemorrhage, which were observed as completely as circumstances would permit. Blood volume tests were made in fourteen cases, and with one or two exceptions the tests were performed repeatedly. The patients were of two types,—those suffering (*a*) from the late effects of primary hemorrhage and (*b*) from secondary blood loss. Infection was present in the majority of instances. The charts of those cases are shown which serve to illustrate best the various findings. Reference only is made to the other cases.

### *A. Initial Changes in Circulation after Hemorrhage.*

#### *1. Blood Volume.*

Blood volume determinations, following hemorrhage, made by the vital red method,[1] on a number of cases showed a surprising reduction

[1] This test was devised by Keith, Rowntree, and Geraghty. It has given very satisfactory results in our hands (Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, xvi, 547).

in the total blood bulk. Patients giving a history of loss of blood when first wounded, with a second hemorrhage several days to a week or more afterwards, as a rule had a volume of 60 per cent or less of the normal. Cases that reached the Base without a positive history of a severe bleeding had not infrequently a diminution in blood volume of 20 to 30 per cent. Text-figs. 1 and 2 (from Cases 1 and 2) are those of patients with secondary hemorrhage.[2] In Case 1 the initial determination, made within an hour after hemorrhage, showed a volume of 3,060 cc.—59 per cent of normal. Case 2 (Text-fig. 2) had a blood volume of 2,663 cc. (estimated by a method described later), or 54 per cent of total volume, shortly after hemorrhage. Case 7 (Text-fig. 7) showed a volume of 3,350 cc., or a reduction to 62 per cent some days after primary hemorrhage. It is not definitely known how low the volume can drop and the patient still live. The lowest figure in this series was 54 per cent. It is possible that lower figures might be obtained in determinations made soon after primary hemorrhage.

With the single exception of blood pressure readings, none of the other data obtained in these cases, such as hemoglobin per cent, findings with the hematocrit, etc., gave any indication of a diminished volume, and it was only after a number of observations on blood volume had been made that we were enabled to associate a lowered pressure with a small blood bulk. The blood pressure, however, is of assistance in judging the volume only when below a certain point. For there may be a considerable reduction of the total blood without any appreciable drop in the pressure. This is due undoubtedly to the fact, now well recognized, that the vasomotor mechanism responds within certain limits to the lessened blood bulk by producing a vasoconstriction which maintains the pressure. Just how great a diminution can occur before the blood pressure falls cannot be stated, and there would seem to be some variation with the individual case. In the present series we have had patients whose volume was reduced to 70 per cent of normal, yet a normal blood pressure was still maintained. Such patients probably had some fall in pressure immediately after hemorrhage, since a blood loss of the amount indicated, unless

[2] The text-figures with their legends will be found at the end of Paper II.

very gradual, practically always causes a drop. But the compensatory mechanism was able to restore the pressure to normal. The patients who had a reduction in volume below 70 per cent showed, with one exception, a blood pressure below normal, the decrease in blood pressure corresponding roughly with the amount of reduction in blood volume. The cases with a volume of 54 to 60 per cent of the normal showed systolic blood pressures of 70 to 80 mm. of mercury. One alone had a pressure of 90 mm. It is somewhat difficult to compare these figures, as the readings were not taken at the same intervals after hemorrhage, some being taken immediately and others after many hours or days. However, low blood pressure has been found to be associated so constantly with reduced volume that it seems fair to draw certain general inferences from these observations. We have come to feel that in hemorrhage cases when a blood pressure is below 95 mm. the blood volume is probably under 70 per cent. With a blood pressure of 80 mm. or less the volume is probably only 60 per cent or less. It is possible that a larger series of cases will show that these estimates need modification and that even more accurate inferences can be made from the blood pressure changes.

Patients suffering from acute blood loss and considerably diminished volume showed a much reduced pulse pressure, to 20 mm. or less. Other cases in which the hemorrhage had taken place more slowly or some time previously had a pulse pressure not under 30 (Text-figs. 3 and 5), although the blood volume was considerably lessened and the blood pressure lowered.

### *2. Amount of Blood Loss or Actual Degree of Anemia.*

The actual amount of 100 per cent hemoglobin blood remaining in the circulation was calculated by multiplying the hemoglobin per cent[3] by the blood volume, and dividing this figure by the normal blood volume (obtained later).[4] This gave the total per cent of hemoglobin—assuming that the patient's hemoglobin normally is

[3] Hemoglobin tests were made by the Palmer method (Palmer, W. W., *J. Biol. Chem.*, 1918, xxxiii, 119), which has been of great value in this study. We agree with Palmer that it is accurate to within at least 1 per cent, and we have often made repeated readings to ½ of 1 per cent.

[4] The normal blood volume could also be calculated from the weight.

100 per cent. Estimations made in this way showed that many of these patients have lost more blood than is commonly supposed. Two markedly anemic patients had a reduction of total hemoglobin to 16 per cent of normal (Text-figs. 4 and 5). These cases, however, both gave a history of the first hemorrhage occurring several weeks before. Case 2 (Text-fig. 2) had his initial hemorrhage only the day before and showed a reduction to 35 per cent. Case 7, with a history of only one hemorrhage, showed the same reduction. Other patients showed a reduction of 60 to 75 per cent of their total hemoglobin. How much they lose with a single hemorrhage it is not possible to estimate, since one cannot be sure that they have bled only once. The cases showing a marked reduction in total hemoglobin must have bled repeatedly. These results show that with a remarkably small amount of hemoglobin the circulation can supply enough oxygen to the tissues to keep the patient alive. It seems probable that a reduction much below 16 per cent would be incompatible with life.[5] Both the patients showing this low figure were critically ill.

### 3. *Distribution of the Blood in the Vessels.*

Numerous hemoglobin estimations made on blood from the ear and vein taken simultaneously have shown a definitely higher hemoglobin per cent on the capillary side when the blood pressure is low (Text-figs. 5, 6, and 9). The greatest difference in the two readings has been 8 points. The degree of discrepancy does not seem to parallel the extent of blood pressure drop in the different cases, since in the case showing 8 points difference between ear and vein the blood pressure was only 95 mm., while in Case 9 (Text-fig. 9) the difference was 6 points with a blood pressure of 65 mm. However, repeated readings in the same case show that the degree of discrepancy between ear and vein hemoglobins does have a relation to the blood pressure, since this discrepancy diminishes as the pressure rises. We have come

[5] This is supported by the findings of Rous and Wilson (Rous, P., and Wilson, G. W., *J. Am. Med. Assn.*, 1918, lxx, 219) in animals. They found that an abrupt reduction of the total hemoglobin to one-quarter of the original normal was well tolerated when blood volume was maintained.

to regard these readings as giving valuable evidence on the state of circulatory efficiency. A difference of 6 to 8 points between the capillary and venous blood must mean a considerable stagnation of red cells with a consequent diminished oxidation. It is apparently not necessary that a marked drop in blood pressure should occur before the capillary and vein hemoglobins begin to differ.

### *B. Progressive Changes in the Circulation after Hemorrhage.*

#### *1. Blood Volume.*

*Methods Employed for Estimating Volume Changes.* (*a*) *Vital Red.*—Repeated vital red tests were made on a number of cases (Text-figs. 1, 2, 4, and 7).

(*b*) *Gum Acacia.*—Blood volumes were also estimated from the drop in hemoglobin per cent produced by the intravenous injection of a known amount of 6 per cent gum acacia solution. Text-fig. 3 illustrates the use of this method. Case 3 had a hemoglobin of 61.5 per cent. After the injection of 580 cc. of gum acacia solution the hemoglobin fell to 52.5 per cent. This was a drop of 9 points, or 14.6 per cent of 61.5. With the addition of a colorless diluent to a colored fluid the drop in color should be in inverse proportion to the increased volume of the solution; therefore taking 61.5 per cent as 100, the formula for estimating the new volume would be

$$100 : 14.6 :: x : 580$$
$$14.6\ x = 58{,}000$$
$$x = 3{,}973$$

Subtracting 580 from 3,973 gives the volume before the gum injection. Estimations made with vital red at the same time showed a volume of 3,844 cc., which would give an error of 3 per cent to the gum acacia estimations.

Blood volume estimations might be made in this way with other kinds of colorless fluids injected intravenously. However, gum acacia solution is particularly well adapted to this purpose, since it is retained in the blood stream, while the majority of other solutions are rapidly eliminated from the circulation.

(*c*) *Hemoglobin Changes.*—Blood volume changes were estimated in a third way by changes in hemoglobin per cent occurring as a re-

sult of fluid absorption into the circulation. The approximate increase in the blood bulk can be calculated in this manner only if the blood volume has been already determined by one of the two methods just mentioned. Such a starting-point is necessary. Case 7 (Text-fig. 7) shows the method by which the estimation is carried out. This patient had a drop in hemoglobin from 48.5 to 38.5 per cent occurring in the course of 24 hours, which equals a fall of 20.6 per cent of his original hemoglobin. His blood volume before the drop occurred was 3,665 cc. One uses the same formula as for the gum acacia volume estimation, except that in this instance we know the previous volume; therefore 100 — 20.6, or 79.4 is taken as the new hemoglobin per cent. Then

$$100 : 79.4 :: x : 3{,}665$$
$$79.4\,x = 366{,}500$$
$$x = 4{,}617$$

A second vital red estimation done at this time gave a volume of 4,715 cc. This difference of 100 cc. can be accounted for to a certain extent by the increased production of erythrocytes as shown by the presence of 8 per cent reticulated cells. Even though the error were several times as great as this—and it may be said that the calculation did not work out so closely as the above in all cases—the method is still of value.

*Effect of Transfusion and the Injection of Gum Acacia.*—In the present series of cases transfusions were given with both citrated blood and whole blood (paraffin tube). The blood volume was increased by both procedures, and the increase persisted equally well following either method. The effect of gum acacia injections was entirely similar in this respect. However, gum acacia solution was not used in very anemic patients. The most interesting fact revealed by the repeated blood volume tests was that transfusion or infusion only begins the work of restoration of the blood bulk. Case 2 (Text-fig. 2) is a good example. The estimated initial volume in this case was 2,660 cc., or 54 per cent of the normal. Transfusion of 800 cc. of blood increased the volume to 3,460 cc., which was still 1,440 cc. below the patient's normal volume. In other words, even after transfusion the volume was only 70 per cent of normal. It is obvious that in such

cases the volume must still be increased considerably before we can say that the circulation has been restored to a state of normal efficiency. The lacking fluid bulk is made up by the patient from his own body fluids. It has been found necessary in practically all cases to increase the fluid intake in order to bring this about promptly. By this means, in the patient referred to (Text-fig. 2) the volume was increased progressively to normal during the $2\frac{1}{2}$ days following transfusion. (This was indicated by the drop in hemoglobin to the lowest level reached on April 30 and was shown to be the fact by a vital red test made the next day.) This case is typical of others studied in the same way. Text-fig. 3 shows the same increase in volume after gum acacia solution injection. With the progressive increase in volume after transfusion the hemoglobin falls, which is itself an index of increasing blood bulk. Thus we have come to expect in transfusion cases a drop in hemoglobin and the volume of formed elements (hematocrit) following a preliminary rise. If this does not occur it is taken to signify that the patient's blood is not diluting as it should, or, otherwise expressed, is not receiving the expected fluid increment from the tissues; and an increased fluid intake is indicated. When the hemoglobin per cent drops and then gradually becomes stationary, the volume has probably reached a level well up toward normal, provided the fluid intake has been good. An exception to this is to be found in the cases of well marked anemia discussed later.

### *2. Relation of Blood Volume to Hemoglobin Per Cent.*

The extent to which the blood becomes diluted through a restoration of its volume after hemorrhage seems to depend within certain limits on the total amount of hemoglobin remaining. We have observed in patients with a very low hemoglobin per cent that the blood volume does not show a marked increase until the amount of hemoglobin rises, no matter how much fluid they are given. There would appear to be two forces at work in these cases—one which tends to restore the blood bulk so that the circulation has a sufficient volume to work with effectively, and the other a mechanism tending to inhibit dilution of the blood with the consequent lowering of the

hemoglobin per cent thus entailed. Until a very low per cent of hemoglobin is reached by bleeding, the former force is the stronger, and the blood dilutes to the volume needed for a good blood pressure. There seems to be a point at about 20 per cent hemoglobin where the latter force is the more effective and the blood does not dilute—which would reduce the hemoglobin below this point—even though the volume may be so small as to be barely compatible with life. Case 4 (Text-fig. 4) showed a condition of profound anemia—hemoglobin 20 per cent. A fairly large fluid intake was maintained, yet the blood showed no tendency to dilute, even though the volume was less than 68 per cent of the normal. Case 5 (Text-fig. 5) shows a first hemoglobin reading of 26.5 per cent and 30 per cent from vein and ear respectively. At this time the blood volume (estimated) was 2,605 cc., or 56 per cent of normal. This volume was evidently too small for the circulatory needs, and dilution was permitted to occur until the hemoglobin reached 23 per cent. At this point the hemoglobin remained practically stationary, the volume having increased meanwhile sufficiently to bring the blood pressure up to normal.

When this balance between the opposing forces of blood dilution and blood volume has been reached, any further increase in volume seems to be dependent on an increase in hemoglobin. In Case 4 (Text-fig. 4) it is seen that the blood volume stayed low until the 23rd; then, coincident with a well marked increase in hemoglobin—due to the tremendous bone marrow stimulation present, as shown by the great number of reticulated cells—the volume took a decided rise. The same phenomenon is seen in Case 1. Following the second transfusion on April 9, the volume remained as before at about 4,300 cc., then showed a tendency to drop off (the patient had marked sepsis at this time, and an amputation was performed). During this period the hemoglobin stayed at a level of 38 to 39 per cent. The production of new red blood cells, as indicated by the number of reticulated elements, had dropped almost to normal. On April 14 a marked stimulation of the bone marrow must have occurred, for the per cent of reticulated cells rose rapidly until it had reached a high figure. Coincident with this increase in blood production the blood volume began to increase, and by the 20th, if not sooner, the

volume had reached normal. Even though a volume estimation is lacking here, the fact that there was no rise in hemoglobin per cent in spite of the great increase in blood production would have led one to infer that the total blood bulk must be increasing.

It would seem from the observations in the present series of cases that in the absence of severe complications, as sepsis, shock, etc., this failure of the volume to return approximately to normal occurs only when the hemoglobin is below 40 to 50 per cent. In patients with more hemoglobin than this the volume increases to somewhere near normal very promptly. In one or two cases of marked sepsis the rule did not hold.

This finding indicates definitely the beneficial effect of transfusion in patients in whom there is a marked reduction in total hemoglobin. By the addition of new red cells to the circulation the organism is enabled to restore the blood volume to a much greater extent than would have been possible without transfusion. In other words, by means of transfusion we have taken the patient out of the class of limited blood dilution and put him into the class in which well marked blood dilution can occur.

### *3. Relation of Blood Pressure to Hemoglobin Per Cent.*

The cases with the most marked anemia, uncomplicated by any noteworthy sepsis, have shown an increase in blood pressure to above normal when the blood volume has been increased. This high pressure has persisted until an increase in hemoglobin occurred. The abnormally high blood pressure has been accompanied by an increased pulse rate as well. Case 4 (Text-fig. 4) is the best instance, as this was the most anemic patient of the series. As long as the hemoglobin remained at 25 per cent or under the blood pressure varied from 135 to 145 mm. with a pulse rate of 140. With a rise in hemoglobin the pressure fell to normal where it remained. The pulse rate showed a parallel drop, though it did not reach normal. Text-fig. 1 shows the same phenomenon. After the second hemorrhage on April 6, which was followed by a drop in hemoglobin per cent to 26, the pressure rose to 145 mm. and remained high until after transfusion which increased the hemoglobin to 38 per cent. The pulse

followed the general curve of the blood pressure but did not show a parallel drop, owing, no doubt, to the presence of marked sepsis at this time.

There seems reason to believe that this increase in blood pressure and pulse during the period of marked anemia is a compensatory reaction, which takes place in order to produce a more rapid blood flow and thereby a more effective utilization of the small amount of circulating hemoglobin. As might be expected, this increased blood pressure did not occur when the volume was very much reduced. The relation between blood volume and blood pressure has already been discussed.

*4. Changes in the Hemoglobin of Blood from Capillary and Vein.*

In cases showing a difference in hemoglobin per cent between the blood of capillary and vein, we have found that as the pressure rises with the increase in volume, this discrepancy diminishes progressively until the two readings finally become the same, indicating that an even redistribution of the red blood cells has been effected (Text-figs. 5, 6, and 7). Case 9 (Text-fig. 9) shows further the relation between the blood pressure and the amount of the difference between the hemoglobin of ear and vein. In this patient a secondary drop in blood pressure was accompanied by an increased separation of the two hemoglobin readings. It is interesting to note also in these cases that a considerable rise in blood pressure is necessary to bring the two readings together. On Text-fig. 6 it is seen that even after transfusion, when the blood pressure had reached 105 mm., the ear and vein hemoglobins were still 2 points apart. Later, following dilution of the blood and a rise in pressure to 110 mm., there was a separation of only 1 point between ear and vein hemoglobin, a difference which is practically negligible. In Case 7 with the blood pressure at 93 mm. there was a separation in ear and vein hemoglobins of 6 points. In this case also the two readings had not come together until the blood pressure reached 110 mm.

A diminishing difference between ear and vein hemoglobin may be taken as a decidedly favorable indication, even though there has not been much change in blood pressure, since it seems to show even

more accurately than the blood pressure the direction the case is taking. It is possible that this is a more delicate indication of a beginning increase in blood volume than is the blood pressure. A lack of such a diminishing difference, on the contrary, may be regarded as an unfavorable sign. This last was observed by Cannon and his coworkers[6] in shock cases. The hematocrit readings made on ear and vein blood have been found to parallel the hemoglobin changes.

*5. Blood Production.*

We have made reticulated red cell counts in all these cases at frequent intervals, usually daily. The rapidity of increase and the height to which the per cent of these cells rises seem to depend largely on the degree of anemia present. In other words, the strength of the stimulus controls the degree of increase in blood production. In the markedly anemic cases the number of reticulated cells rises rapidly so that within 24 hours after hemorrhage the blood production may have increased to many times normal. Text-fig. 4, Case 4, shows, the day following a marked hemorrhage, reticulated cells at 5.2 per cent. By the next day they had increased to 15 per cent, and the rise continued until it had reached 25 per cent on the 7th day. The reason for the tremendous bone marrow stimulation may be interpreted here as the very low hemoglobin, beginning at 20 per cent. A contrast to this case is afforded by Text-fig. 3. The patient showed a moderate degree of anemia, with a hemoglobin of about 50 per cent. The reticulated cells increased much more slowly and finally reached only 8 per cent, or about one-third the highest per cent of Case 4. The stimulus in this instance was very much less.

As soon as the increase in hemoglobin occurs, the per cent of reticulated cells falls. This is shown well by Case 5. With a hemoglobin at 23 per cent the reticulated cells numbered 12 per cent. At this point the patient was transfused. The hemoglobin rose to 40 per cent, and the reticulated cells fell to approximately half their number. Text-fig. 4 also showed a rapid drop when the hemoglobin rose over 30 per cent.

[6] Cannon, W. B., Fraser, J., and Hooper, A. N., *J. Am. Med. Assn.*, 1918, lxx, 526.

There seems to be another factor which influences the production of new blood in these cases, and that is the dilution of the blood with the restoration of the volume after hemorrhage. Reticulated red cell counts were made on an earlier series of hemorrhage cases before particular attention was paid to the restitution of blood volume. In these cases no attempt was made to restore the fluid bulk of the circulation by increasing the fluid intake, and the lack of more than a slight drop at most in hemoglobin and red cell count in these patients after transfusion or after hemorrhage when not transfused, showed that the blood volume was being made up only slowly. The percentage of reticulated cells did not go much over 3 to 4 per cent in any of the patients and usually there was very little increase over normal. Further, when the increase did occur it was much slower than in the present series of cases in whom the volume was made up rapidly. Text-fig. 7, Case 7, shows the effect of volume restoration on blood production. The patient had the primary hemorrhage 1 week previous to the first observations, and at the time of these the reticulated cells were only 2 per cent. With the restoration of the blood volume the number of reticulated cells increased rapidly so that at the end of 24 hours they had reached 8 per cent. The reason for the relatively great percentage of reticulated cells in the present series of cases is not altogether clear. However, a possible explanation lies in the fact that in them the hemoglobins were lower on the average than in the first series, owing to blood dilution consequent on restoration of the blood volume. The diminished hemoglobin per cent per unit of blood may act as the needed stimulus to the bone marrow.

Text-fig. 4, Case 4, illustrates the value of reticulated red cell estimations as an aid to prognosis. On account of the presence of intestinal hemorrhage, transfusion in bulk was considered inadvisable, since it was thought possible that an increase in blood pressure might start bleeding. The patient was in a very critical condition, with a hemoglobin of 20 per cent, and repeated small transfusions were considered. The rapid rise in the number of reticulated cells, however, led us to believe that the patient might take care of the hemoglobin deficiency himself, provided no more bleeding occurred. Two small transfusions only were given. As can be seen on the chart, blood production occurred very rapidly—except for a pause

from the 21st to the 23rd due to possible fresh bleeding—and by the end of 6 days the patient had practically doubled his total hemoglobin.

*Blood Destruction.*—In order to determine whether or not increased blood destruction was occurring, spectroscopic tests for hemoglobin and urobilin were made on the urine in all the patients studied. No evidence of increased blood destruction was found in any of these cases.

*Blood Groups.*—Compatible donors were used for transfusion in every instance. With one or two exceptions the donors belonged to Blood Group IV. No reactions occurred after transfusion.

### *6. Hematocrit Tests.*

Successive hematocrit readings were made on most of the cases at frequent intervals. The initial results with the hematocrit were found to parallel the hemoglobin percentages, and the later findings showed practically corresponding variations. However, when blood production began to increase, the increase in cell volume, as shown by the hematocrit, rose above the hemoglobin; *i.e.*, the color index became less than 1. This difference became well marked during the middle period of regeneration, then tended to diminish in most instances as the hemoglobin reached 70 to 80 per cent. The patients could not be kept long enough to observe when the color index rose to 1 again. The repeated hematocrit readings served as a check on the hemoglobin per cent, but aside from indicating a low color index yielded no additional information.

## SUMMARY.

Blood volume tests made by the vital red method (Keith, Rowntree, and Geraghty) on patients after hemorrhage showed a marked reduction in the total blood bulk. Not uncommonly the blood volume was less than 60 per cent of the normal. The reduction after a certain point had been reached seemed to parallel the decrease in blood pressure. This relation of diminished blood volume to low pressure suggested a rough method of estimating blood volume from the change in blood pressure.

By means of the blood volume and the hemoglobin per cent the actual amount of blood loss was determined. Cases of severe anemia showed a loss of as much as five-sixths of their total hemoglobin.

Progressive changes in blood volume following hemorrhage were estimated in three ways: (1) repeated vital red tests; (2) calculation from changes in hemoglobin per cent produced by the injection of gum acacia; (3) calculation from changes in hemoglobin per cent following the dilution of the blood by the patient's own body fluids.

The effects of the different methods of transfusion and of injection of gum acacia on blood volume were observed. No differences were apparent. It was found that transfusion and gum injections only partially restored the blood volume. Forced fluids by mouth were found to bring about its complete restoration in a comparatively short time.

It was observed that the organism did not restore its blood volume beyond a certain point when a further increase in it would, by dilution, have brought the hemoglobin per cent to a very low figure. In such cases a further increase of the blood volume occurred only when the hemoglobin rose. In cases with a low hemoglobin per cent as the result of a restoration of the blood bulk an abnormally high blood pressure appeared, which continued until the hemoglobin per cent again increased.

Accompanying the low blood pressure seen shortly after hemorrhage was a well marked difference in hemoglobin per cent between capillary and venous blood, with a relative concentration on the capillary side. As compensation occurred and blood pressure rose this difference lessened until the two readings were identical, indicating an even redistribution of the red blood cells.

Reticulated red cell counts made in these cases showed that a marked bone marrow stimulation occurs after hemorrhage. However, except in the very anemic cases the degree of increased blood production seemed to depend largely on the restoration of the blood volume. The patients who were put on forced fluids, with consequent rapid restoration of blood volume, showed a much higher per cent of reticulated cells than those in whom no attempt was made to increase the amount of fluid in circulation.

# BLOOD VOLUME IN WOUNDED SOLDIERS.

## II. The Use of Forced Fluids by the Alimentary Tract in the Restoration of Blood Volume after Hemorrhage.

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(Received for publication, October 3, 1918.)

### *Slow Rate of Blood Dilution Following Hemorrhage.*

Observations made recently by several workers have led them to infer that after hemorrhage in wounded soldiers the blood volume is restored relatively slowly. Govaerts[1] found that, following primary blood loss, the red cell count decreased progressively till about the end of the 3rd day, when the posthemorrhage anemia became fully established. He regarded this finding as indicative of a correspondingly gradual dilution of the blood. Others have drawn much the same conclusion from estimations of the hemoglobin per cent after hemorrhage, though from less complete data. We have found by direct blood volume tests that a small blood volume often persists for relatively long periods after hemorrhage. Patients who had been wounded several days before admission (at the Base) and had no secondary hemorrhage still showed a diminished blood volume. Case 3 (Text-fig. 3), wounded about 24 hours before the blood volume was determined, had a total blood bulk of only 3,265 cc. This low figure showed that there could not have been much restoration of the blood bulk since the primary hemorrhage. Case 2 (Text-fig. 2) was wounded about 36 hours previous to our observations. During

[1] Govaerts, P., *Ambulance Ocean*, 1917, i, 355.

operation he had a hemorrhage which was soon controlled and he could not have lost more than a few hundred cubic centimeters at most, yet his estimated volume at this time was 2,660 cc., or 54 per cent of normal. This means that his blood volume must have been low before the secondary hemorrhage. Other cases have shown a diminished volume at a much longer time after the primary hemorrhage. Case 7, as shown in Text-fig. 7, had, 7 days after the primary hemorrhage, a blood volume of only 3,350 cc., or 62 per cent of the normal volume. Another patient gave no history of secondary hemorrhage, yet 9 days after he was wounded the blood volume was only 3,000 cc. Again, a man wounded 6 days previous to the blood volume test showed a volume of 2,650 cc. The restoration of volume in the last three instances may have been slower than is usually the case, but at any rate it would seem that in wounded soldiers several days usually elapse after a severe hemorrhage before the blood bulk is made up.

The lack of prompt dilution of the blood after hemorrhage would seem to be due chiefly to an insufficient quantity of reserve fluid in the tissues, since it is from this reservoir that the circulation obtains new supplies of plasma. In other words, the body fluid reserves are low in wounded men, and they must be made up before the blood volume can increase. There may be other reasons why the dilution of the blood is delayed; but the striking fact is, as we have found, that if these patients are given large quantities of fluid by the alimentary tract their blood volume increases rapidly.

### *Effect of Forced Fluids on Blood Volume.*

We were first led to try the effect of forcing fluids by finding that even after transfusion the blood volume was still far below the normal and remained low for some time. One patient who had a blood volume of 2,723 cc. before transfusion showed an increase in volume to only 3,886 cc. 8 days afterwards. However, when such a patient was put on forced fluids—water by mouth, and in certain cases saline solution by rectum as well—the volume showed a surprisingly rapid increase which was maintained. Text-fig. 2 illustrates well the effect of a large fluid intake. In this case the volume did

not rise to any extent until the fluid intake had been markedly increased. During the 1st day after transfusion the intake was only 1,000 cc., with the result that the volume rose only 150 cc. The succeeding day, however, forced fluids were begun and the patient took in 2,750 cc. with a consequent marked rise in volume which was still further increased by the following day, when it reached normal. It is interesting to note that thereafter in this case although the large fluid intake was maintained for several days, the hemoglobin could not be forced below 53.5 to 55 per cent, which indicated that the volume had been restored.

Case 8 (Text-fig. 8) was put on forced fluids about 6 hours after transfusion. Unfortunately the hemoglobin could not be obtained before or immediately after transfusion. At the time the fluids were started the hemoglobin reading was 66 per cent. During the course of the next 22 hours the patient took in a large quantity of fluid, both water by mouth and normal salt solution by rectum, and the hemoglobin per cent showed a drop of 20 points, indicating a marked increase in blood bulk. Volume tests were not done in this case.

*Fluid Intake and Output.*—We have a further indication that the fluid reserves are low in these cases from the fact that the output of urine was at first very small compared with fluid intake. In Case 8 the intake during the first 22 hours following transfusion was 4,400 cc., while the output was under 800 cc., or less than one-fifth the intake. This marked diminution of relative fluid output occurs only while the volume is increasing rapidly. As soon as the volume has been made up, the relation of intake to output returns to the normal proportion, roughly speaking of 3:2. In Case 7 (Text-fig. 7) the intake during the first 24 hours was 4,800 cc., the output only 770 cc., approximately 6 : 1. During this time the blood volume had increased from 3,350 cc. to 4,715 cc. In the following 24 hours the intake was 3,850 cc. and the output 1,800, about 2:1. The volume showed only a slight increase during this time—100 cc. The next day the output was almost as large as the intake. The volume by this time was approximately normal.

*Rapid Absorption of Fluids Given in Large Quantities.*

*In the Treatment of Hemorrhage.*—The prompt way in which fluids taken into the alimentary tract caused an increase in volume of the blood led us to wonder whether hemorrhage cases might not be treated by this means alone. Even if an increase of only a few hundred cubic centimeters of plasma could be brought about within an hour or so, this might be sufficient to tide the patient over the critical point, provided his blood pressure began to rise very soon.

The results in the first case treated in this way are shown in Text-fig. 5. This patient had three hemorrhages in 12 days. Following the third, which was not very severe, the blood pressure was somewhat below 90 mm. of mercury. When the first observations were taken the patient was on the operating table and his arm was being manipulated. The pressure at this time was 75 mm. The first hemoglobin observations were made at 7.35 p.m.: ear hemoglobin 30 per cent; vein 26.5 per cent. This difference showed a good deal of circulatory insufficiency in view of the fact that the hemoglobin per cent was at such a low figure. The systolic blood pressure was then 90 mm. Forced fluids were started an hour later. At 10 p.m. he had another small hemorrhage, which was shortly controlled but caused a drop in pressure from 95 to 80 mm. However, the pressure recovered fairly quickly and by 3 a.m. had reached 105 mm. The pulse had dropped from 140 to 115. By 9 a.m. the next morning the patient had taken 3,000 cc. of fluid, chiefly by mouth, the blood pressure had risen to 115 mm., the hemoglobin had fallen to 23 per cent, and the ear and vein blood gave the same reading. The estimated volume increase during the night was 600 cc. In the course of the day the blood pressure showed a downward tendency, and the ear and vein hemoglobin became separated by 1 point. This is not, however, outside the margin of error. The patient was so anemic that in spite of a high per cent of reticulated red blood cells transfusion seemed indicated. His total hemoglobin was only 15.9 per cent of normal. The hemoglobin rose to 40 per cent after transfusion. A vital red test made just after transfusion gave a volume of 4,010 cc. Subtracting 800 cc. of fluid introduced would give him a volume of about 3,210 cc. before transfusion.

Although transfusion was finally performed, it seems evident that forcing fluids after hemorrhage tided the patient over the immediate emergency, by increasing his volume sufficiently to bring the pressure up to normal. Unfortunately the fluid output was not recorded. The patient did well for 7 to 8 days, when his arm became septic. A vital red test at this time showed the same volume as before. This condition of sepsis persisted for some time, then finally cleared up, and the hemoglobin which had remained almost stationary began to rise markedly. The final volume was 4,675 cc. It was felt afterwards that the blood pressure might have been raised more rapidly in this case had the fluids been pushed more energetically at first. In the subsequent cases larger amounts of fluids were given in the first few hours. It was found that these patients could absorb saline enemata at relatively short intervals. Continuous rectal drips were also used.

Case 7 (Text-fig. 7) illustrates the effect of a more intensive fluid administration. This patient had bled 7 days previously and was apparently doing well at the Casualty Clearing Station. When first seen on June 2, 10 to 12 hours after a long ride on an ambulance train, he had a systolic blood pressure of 93 mm., a diastolic pressure of 68 mm., a feeble pulse though not rapid, an ear hemoglobin of 56 per cent, and vein hemoglobin of 50 per cent. He showed no evidence of sepsis, and it was felt that his poor circulatory condition was due to a low blood volume rather than to any actual hemoglobin lack. At 11.10 a.m. forced fluids were started,—all the water he could drink and saline enemata of 250 cc. (8 ounces) every hour. Within half an hour he had taken 900 cc. His blood pressure had increased to 96 mm. At the end of an hour the intake was a little over 1,000 cc. The blood pressure was 98 mm. Within $1\frac{1}{2}$ hours he had taken 1,150 cc.; his systolic blood pressure had reached 106 mm. and diastolic 70 mm., an increase in pulse pressure from 25 to 36 points. The ear hemoglobin had dropped to 49 per cent and the vein to 47 per cent—a difference now of only 2 points, showing an improvement in the circulation coincident with the increase in blood pressure. 2 hours later, that is $3\frac{1}{2}$ hours from the time the fluids were started, the patient had taken in 1,664 cc., the systolic blood pressure was 110 mm., the pulse pressure was 35 mm., and the ear and vein hemo-

globin read the same—48.5 per cent. A vital red estimation made at this time showed a volume of 3,665 cc. Calculation of the increase of volume from the drop in hemoglobin gave a beginning volume of 3,350 cc. (62 per cent of normal). The average vein-ear estimation beforehand was 53 per cent, the drop was 4.5 points, or 8.5 per cent of 53 per cent, and therefore

$$100 : 91.5 : : 3{,}600 : x$$
$$x = 3{,}350$$

An operation was then performed. The blood pressure showed a marked rise under the anesthetic to 148 mm., with a subsequent drop to normal. Forced fluids were continued. By 7.30 a.m. the following morning the intake had reached 4,800 cc., the output was 768 cc., the hemoglobin had fallen to 39 per cent, and the reticulated cells had increased from 2.4 to 8 per cent. A second vital red estimation showed a volume of 4,715 cc. The marked deficiency in blood volume and hemoglobin which had existed at first were now strikingly apparent. The hemoglobin remained practically stationary now for 24 hours, while the blood volume showed a gradual increase which could be accounted for largely by the high per cent of new cells. The reason the volume did not increase to normal at once is probably that given in Paper I;[2] namely, the action of a certain adjusting mechanism whereby very anemic blood is not permitted to undergo further dilution after a sufficient fluid bulk has been put in circulation to give a good blood pressure. The explanation for the subsequent drop in volume from June 6 to 9 is not clear. As regeneration progressed there was a wide separation of the hematocrit and hemoglobin readings which came together again at the end of 4 weeks.

*As Indicating Degree of Blood Loss.*—It has been pointed out, in connection with the cases just described, that the degree of dilution of the blood when fluid is forced gives an index of the amount of blood lost. In all these cases, however, the initial hemoglobin per cent was moderately reduced when the patient was first seen, indicating that considerable hemorrhage had occurred, although it told us nothing as to the amount. There is another type of case in which it is impossible to tell by looking at the patient whether there has been any appreciable

[2] Robertson, O. H., and Bock, A. V., *J. Exp. Med.*, 1919, xxix, 139.

hemorrhage. The hemoglobin does not help since it is approximately normal. The blood pressure is low, but that is said to be due to shock. A case of this type is shown in Text-fig. 9. This patient had been run over by an engine; both legs were crushed. It could not be ascertained how much he had bled. His ear hemoglobin was 94 per cent, and vein hemoglobin 88 per cent; the systolic blood pressure was 65 mm. The general opinion was that he was suffering principally from shock. He was put on fluids by rectum shortly after admission, but these were not pushed energetically enough. At the end of 2 hours operation was considered indicated, and as the pressure had not risen appreciably, he was given 550 cc. of gum acacia. A double amputation was done. During this time he had received 500 cc. of saline solution by rectum—a total of 1,050 cc. of fluid. The hemoglobin was again taken and read 61 per cent vein and 63.5 per cent ear. Fluids were kept up and by 5 p.m. the following day the hemoglobin had fallen to 50 per cent with the ear and vein readings the same. During this time, about 33 hours, he had taken in 5,720 cc. of fluid and put out only 832 cc. For the first 22 hours he passed no urine. The only interpretation possible for this marked drop in hemoglobin from over 90 per cent to 50 is that the patient must have had a marked hemorrhage at the time of injury, losing almost half his total blood. Although there may have been shock in this case, and there probably was, yet his symptoms, low blood pressure, collapse, etc., could all have been due to the tremendous and rapid reduction in blood volume.

### *Indications for, and Use of Different Methods of Treatment in Hemorrhage.*

*Estimation of the Actual Condition of the Circulation.*—In attempting to determine a patient's condition after hemorrhage with a view to deciding on the most suitable form of treatment, it is of the greatest importance to think of the state of the circulation in terms of absolute blood loss. The blood volume determinations have shown that hemoglobin per cent tells one relatively little in most cases, since there may be only a moderate lowering of the hemoglobin reading, perhaps to 55 or 60 per cent, but with a volume diminished to less

than 60 per cent of normal, so that the existing anemia is really marked. Again the hemoglobin may be much reduced, say to 30 per cent; and while a patient can get along with 30 per cent hemoglobin if his blood volume is good, with a blood volume much diminished he may be in a dangerous state of anemia. It is true that blood volume tests cannot generally be made in hemorrhage cases for obvious reasons. But the information obtained from these tests performed in a number of patients has given us a conception of what actually occurs after blood loss, and from these findings we have been enabled to associate diminished volume with the changes in blood pressure described in Paper I.[2] By means of this rough method of estimating blood volume, one is able to obtain a general idea of the total blood loss. For example, with a hemoglobin of 40 per cent and a blood pressure below 80 mm., one may estimate that the total hemoglobin remaining in the circulation is only about 60 per cent of 40, or 24 per cent at most, and that the patient needs transfusion, since his oxygen-carrying capacity is very low. If the hemoglobin was 60 per cent and the blood pressure the same as above, *i.e.* 80 mm., one could estimate the total hemoglobin at about 60 per cent of 60, or approximately 36 per cent. We have shown that a patient can get along quite well with 36 per cent total hemoglobin, provided his volume is sufficient to maintain a good pressure. Thus the chief need in a case of this kind is increased blood bulk and not new red blood cells.

*Methods Employed.* (*a*) *Transfusion.*—As already pointed out, the principal indication for transfusion is the need of more red corpuscles —oxygen carriers. We have come to feel that when the total hemoglobin falls to 25 per cent the infusion of new blood is indicated. Although a patient can live with a total hemoglobin lower than this, his margin of safety is not very great. Another hemorrhage, perhaps even a small one, may be more than he can stand. The restoration of the blood volume must also be considered in this connection. We have seen that in the presence of a much diminished amount of hemoglobin, no matter how large a quantity of fluid is given, the blood volume does not show any marked increase until new hemoglobin is added to the circulation. Furthermore, such a patient is not in a proper condition to combat sepsis, since his tissues are getting only a

bare sufficiency of oxygen. Experimentally Rous and Wilson[3] found that animals made anemic acutely by bleeding did not survive when the total hemoglobin fell to one-fifth of the normal, even though the blood volume was kept at normal. On the other hand, as these investigators showed, a hemoglobin value of one-quarter of the normal was sufficient for body needs provided the blood volume was promptly restored.

(*b*) *Gum Acacia.*—With the total hemoglobin above 25 per cent the form of fluid treatment best suited to the case depends on the severity and urgency of the circulatory condition. If the condition is such that an immediate and large addition of fluid bulk to the blood is demanded, there is no question but that gum acacia fluid should be given intravenously. A falling blood pressure of 70 mm. or less, with a rapidly rising pulse and general distress will indicate this course.

(*c*) *Forcing Fluids by Alimentary Canal.*—There is a large class of hemorrhage cases in which the condition is less urgent than in those just described, yet sufficiently severe to demand prompt treatment. It is in this class of case that forced fluids by the alimentary tract seem to be of particular use. The fluids should be given as rapidly as the patient will take them—water by mouth and normal salt solution by rectum. The patients will retain at least 250 cc. (8 ounces) given by rectum every hour and when the blood loss has been marked this amount can be given every half hour. The forcing of fluids should be kept up for at least 24 hours and better for 48 to 72 hours, so that the fluid reserves are restored as well as the blood volume. This last applies to the cases treated intravenously also. It seems questionable whether non-colloidal solutions, such as salt solution, Ringer's solution, etc., given in other ways, have the same beneficial effect as fluids by the alimentary tract. Salt solution given intravenously has been definitely shown to be eliminated from the circulation very rapidly and often leaves the blood more depleted than before. Whether solutions given subcutaneously would work as well as by rectum is not known. Theoretically with a failing circulation such fluid would be absorbed only very slowly. The peculiar benefit from

[3] Rous, P., and Wilson, G. W., *J. Am. Med. Assn.*, 1918, lxx, 219.

forcing fluids by mouth and rectum is that a fairly large percentage of such fluid appears to be added to the circulation in the form of blood plasma.

We wish to emphasize again that the methods described above of estimating the total blood loss and the indications for its treatment have been applied only to cases of secondary hemorrhage or cases of primary hemorrhage seen 24 hours or more after the blood loss.

## DISCUSSION.

*Significance of Diminished Blood Volume.*—The relation of blood pressure to a markedly diminished blood volume is evident. In order to keep the circulatory system filled, a certain minimum of circulating fluid is necessary. The dangers of a low blood pressure are well recognized, and one of the most obvious procedures in medical practice in such conditions is to introduce fluid of some kind into the circulation in order to raise the blood pressure. But is raising the blood pressure to normal all that is necessary? We have shown that the blood pressure may be normal with a reduction in blood volume of 25 to 30 per cent. Unless the volume is further reduced, there may be no drop in pressure. The adaptive mechanism which enables the vascular system to adjust itself to a small blood bulk in order to maintain a normal pressure must have a limit, and without performing actual blood volume tests one cannot say in any given case how near the limit of such compensation the individual is. Furthermore, the maintenance of a normal pressure with a diminished volume depends entirely on a vasomotor mechanism which is responding well to the increased demands made by these abnormal conditions. The circulatory balance may be on the verge of profound disturbance, but as long as the patient is quiet and at rest the compensation is held. However, if an increased strain is put on a circulation in this condition, such as an anesthetic, operation, infection, or recurrence of blood loss, the vasomotor mechanism may fail and a drop in blood pressure result. We have not had an opportunity to study in detail many instances of this kind but have observed several cases in which there was a good blood pressure with a reduced volume, and in these the reaction to increased strain seemed

to be the result of the circumstances just outlined. An example of this is Case 3 (Text-fig. 3) who had a blood pressure of 105 mm. before operation and a blood volume diminished to about 70 per cent of normal. (The blood volume was not taken before operation, but there is no reason to suppose that it changed much during this brief period as there was very little blood lost except in the amputated limb. The initial volume was probably a little under 70 per cent since the final volume, though not obtained, would probably have been about 4,800 cc.) During the operative procedure the pressure fell rapidly till it reached 70 mm. At this point the patient was given 600 cc. of gum acacia. It would seem in this instance that had the blood volume been a little greater the drop in pressure might have been avoided. If these inferences are substantiated, it is obviously important to restore the blood volume beyond the degree necessary merely to raise the blood pressure.

*Possibilities in the Early Use of Forced Fluids after Primary Hemorrhage.*—All the evidence at hand goes to show that restoration of the blood volume after hemorrhage depends primarily on the content of the body in reserve fluids. When the fluid reserve is low such restoration can take place only to a very limited degree, and the result is a persistent absence of blood dilution. It is probable that after a severe hemorrhage even a normal fluid reserve cannot supply sufficient plasma to restore the blood volume, and additional fluids are needed.[4] The reason for persisting low volumes after primary hemorrhage in the battle zone seems to be due principally to two factors. In the first place, the soldier in the trenches usually has a minimum to drink, and during an engagement he may go for several days with a very small fluid intake. The result is that his fluid reserves become much diminished. A hemorrhage at this time makes a demand for a new supply of fluid for the circulation which cannot be met, or at least met only inadequately. In the second place, after hemorrhage has taken place, no attempt is made as a rule to increase the patient's

[4] Rous and Wilson showed experimentally that after repeated hemorrhage, amounting in all to about half the animal's blood bulk, the tissue fluid reserves were so depleted that the animal could no longer restore its volume. But far greater hemorrhages were withstood when the blood volume was promptly made up.

fluids. He is thirsty and may get a certain amount of water, but often in a rush he gets comparatively little attention, as his great need for increased fluid is not realized. A further factor which undoubtedly tends to diminish the reserve fluid of the body is the profuse sweating so frequently seen in patients suffering from severe hemorrhage.

It is felt by the writers that much can be done in the treatment of primary hemorrhage by the early use of fluids as outlined above. There is no reason to believe that the circulation will not absorb fluids from the alimentary tract after primary hemorrhage, in the manner demonstrated after secondary hemorrhage. Water by mouth and salt solution (possibly even water) by rectum could be started at advanced surgical stations and continued at succeeding stations. The patients might even be supplied with water to drink during the ambulance and train journey, every effort being made to give them as much as possible. It seems probable that if these measures were thoroughly carried out in patients with hemorrhage much so called shock might be avoided, since the long journey from the advanced surgical units to the Casualty Clearing Station must put a very great strain on a circulation hampered by a low blood volume. Also in patients with a tendency to hemorrhage the importance of having the tissues well charged with fluid is evident.

We do not mean to convey the impression that we are in any way advocating forced fluids as a substitute for either gum acacia or blood transfusion. The unique value of these forms of intravenous treatment in appropriate cases is unquestioned. It is as an additional measure that an abundant fluid intake is suggested. Since fluids by the alimentary tract are so easily given, many cases of hemorrhage that cannot be transfused or infused might be aided in this way. Furthermore, in cases of moderate hemorrhage, at least, many transfusions and infusions might be saved if the restoration of the blood volume were begun promptly.

## SUMMARY.

Blood volume tests made on a number of soldiers recovering from hemorrhage have shown that in many instances dilution of the blood occurs very slowly. The principal reasons for this seem to be

(*a*) an initial lack of reserve fluid of the tissues, and (*b*) the absence of any subsequent attempt by the body to make up this fluid deficiency.

By putting such patients on a large fluid intake by mouth and rectum it has been found that their blood volume can be promptly and greatly increased. Hemorrhage cases transfused, yet still showing a low blood volume, were first treated in this way. Then the effect of forced fluids without transfusion was tried. Immediately after a hemorrhage, or as soon as the patient came under observation, he was given large quantities of water by mouth, and salt solution by rectum. Under such treatment the blood pressure soon began to show a progressive rise, the volume increased, and the red cells became more evenly redistributed, as shown by the relative hemoglobin percentages of the capillary and venous blood. These changes were often well marked after only 2 or 3 hours of the treatment.

More than this, forcing fluids in cases where the amount of bleeding is difficult to estimate on account of the presence of a high hemoglobin percentage is of distinct value, since the dilution of the blood which results serves to show the extent of the hemorrhage through the drop in hemoglobin that it entails.

In attempting to determine the condition of the patient after hemorrhage with a view to deciding the most suitable form of treatment, it is of much importance to learn the total blood loss—which is often not even indicated by the hemoglobin concentration of the remaining blood. With a total hemoglobin reduced to 25 per cent or under transfusion is needful. New blood is necessary, not only to supply more oxygen-carrying cells, but also because it actually enables the circulation to increase its volume. For, as has been pointed out in Paper I, the hemoglobin percentage must be above a certain point if a rapid restoration of the blood volume by means of the organism's own activities is to come about. With the total hemoglobin above 25 per cent the chief need is for increased blood volume, and if the patient's condition demands an immediate and large addition of circulating fluid, gum acacia solution should be given. If the condition is not so urgent, forced fluids by the alimentary tract are indicated.

The blood volume can be considerably reduced and yet a normal blood pressure maintained. It is pointed out that the vasomotor

mechanism which has adapted itself to the diminished blood bulk may in any individual case be very near the margin of its compensatory power. Increased strain in such instances may cause a failure of this mechanism with a resulting fall in blood pressure.

The beneficial results of forced fluids after secondary hemorrhage suggest the value of the early use of fluids by the alimentary tract in cases of primary hemorrhage.

We wish to express our appreciation of the help given in this work by Lieutenant Colonel Roger I. Lee, Medical Corps, U. S. Army, through whom the studies were made possible. We are greatly indebted to him for many valuable suggestions and advice concerning certain directions the work has taken. We acknowledge with pleasure the aid given by Major Binney, Medical Corps, U. S. Army, and his staff of surgeons. We are indebted to The Rockefeller Institute for Medical Research for a considerable amount of apparatus. We also wish to thank Colonel T. R. Elliott for his interest and for apparatus obtained through him from the Medical Research Committee.

## EXPLANATION OF TEXT-FIGURES.

TEXT-FIG. 1. Case 1, No. 241,145, age 23 years. Wounded Mar. 3, 1918. Penetrating wound, right thigh, lower third. No history of hemorrhage. Admitted to No. 13 General Hospital Mar. 29. Wound septic. Three hemorrhages during following 9 days; transfusions given after each. Observations begun after second hemorrhage. Developed streptococcus septicemia. Apr. 12. Amputation, right thigh. Sepsis of stump. Slow convalescence. Good recovery.

Shortly after hemorrhage the blood volume (vital red test) was found to be 3,060 cc., systolic blood pressure 80 mm., hemoglobin 42 per cent, hematocrit 17. Immediately after transfusion there was a well marked rise in blood volume, blood pressure, and number of red blood cells. The blood volume continued to increase, while the number of blood cells (red count and hematocrit) fell sharply, indicating blood dilution. A blood volume estimation on the 4th day showed a normal figure. A third hemorrhage occurred at this point, following which the hemoglobin fell to 26 per cent, and the hematocrit to 12. After a third transfusion the hemoglobin rose to 38 per cent, then remained about stationary. The blood volume subsequently fell somewhat. The number of reticulated red cells dropped to almost normal, in spite of the fact that the patient had a well marked anemia (total hemoglobin 31 per cent). This condition persisted until

after the amputation of a gangrenous lower leg, when the number of reticulated cells began to increase rapidly. Accompanying the bone marrow stimulation was a progressive rise in blood volume to normal. The hemoglobin per cent remained stationary until the volume had been restored; the total hemoglobin, however, during this time showed a marked increase.

TEXT-FIG. 2. Case 2, No. 5,442, age 20 years. Wounded Apr. 26, 1918. Severely wounded in thigh (left). Admitted to No. 13 General Hospital Apr. 27. Brisk hemorrhage during operation controlled by ligature of superficial femoral artery. Immediate transfusion of 800 cc. Severe streptococcus infection followed by good recovery.

Immediately after hemorrhage the estimated blood volume was 2,660 cc., the systolic blood pressure 72 mm., the hemoglobin per cent 64, and the total hemoglobin 35 per cent. Transfusion produced a marked rise in blood pressure, the hemoglobin rose to 72 per cent, and the total hemoglobin to 53. A fall in blood pressure occurred 2 hours after transfusion, but was followed by a rise to normal. 12 hours later a vital red test was made, which showed a volume of 3,615 cc. During this time the hemoglobin had dropped from 72 to 68. This would indicate that the volume immediately after transfusion was 3,460 cc. (see formula in text, page 145, for estimating blood volume from hemoglobin changes). The initial volume was obtained by subtracting the amount of blood transfused from this figure. With the much increased fluid intake (begun Apr. 29) the volume increased rapidly to normal and the hemoglobin fell correspondingly. (See page 156.)

TEXT-FIG. 3. Case 3, No. 42,612, age 18 years. Wounded Apr. 17, 1918. Compound comminuted fracture, femur (right), extending into knee joint. Admitted to No. 13 General Hospital Apr. 18 with history of hemorrhage. Amputation through fracture. 580 cc. of gum acacia 6 per cent infused at close of operation. Wound healed without sepsis. Transferred to England May 9 in good condition.

Observations were begun 24 hours after hemorrhage. During operation the blood pressure fell rapidly from 105 to 70 mm. At the end of a short operation the blood volume was 3,265 cc., hemoglobin 61.5 per cent, hematocrit 24. The injection of 580 cc. of 6 per cent gum acacia solution caused a marked rise in blood pressure, and a drop in hemoglobin to 53.5 per cent. The next morning the hemoglobin per cent was the same. A second vital red test at that time showed a volume of 3,844 cc. An estimation of the blood volume from the drop in hemoglobin per cent produced by the injection of 580 cc. of gum acacia gave a volume of 3,925 cc. This agreed fairly closely with the vital red test. With increased fluid intake the volume showed a further rise, and the hemoglobin fell to 45 per cent. After this point the hemoglobin per cent rose progressively. Unfortunately another blood volume test was not made.

TEXT-FIG. 4. Case 4, No. 979, age 25 years. Wounded Apr. 23, 1918. Bullet wound through abdomen. Operated at Casualty Clearing Station 10 hours later. Four perforations of colon, nine of small intestine, two of mesentery. Fecal fistula. Admitted to No. 13 General Hospital May 12. Poor condition. Severe intestinal hemorrhage May 18. Observations begun the following day. Two small transfusions on the 19th and 20th. Satisfactory improvement until development of lobar pneumonia (Pneumococcus Type III) June 4. Died June 10.

Observations were begun 16 to 18 hours after hemorrhage; blood pressure 110 mm., hemoglobin per cent 20, hematocrit 9, reticulated red cells 5.4 per cent. The following day the hemoglobin was 24 per cent, blood volume 2,900 cc., and total hemoglobin 15 per cent. The number of reticulated cells increased rapidly. For 2 days the blood volume and the total hemoglobin rose. Then a drop occurred in both these values, due probably to recurrent bleeding. However, with the tremendous increase in blood production (reticulated cells 25 per cent) the total hemoglobin began to rise again sharply, and at the same time the blood volume increased. Within 3 days the total hemoglobin had been almost doubled. As the need for new blood decreased the percentage of reticulated red cells fell rapidly. An abnormally high blood pressure persisted during the period of most marked anemia. The course of restoration of the circulation was unfortunately interfered with by the onset of pneumonia. (See pages 147 and 148.)

TEXT-FIG. 5. Case 5, No. 862, age 27 years. Wounded Apr. 10, 1918. Severely wounded in arm (right). Admitted to No. 13 General Hospital Apr. 20 from Ambulance Transport because of hemorrhage. Wound septic. Hemorrhages Apr. 21 and May 2. Transfusion May 3. Prolonged sepsis, streptococcus. Transferred to England June 23 in good condition. This case is described fully in the text, page 158.

TEXT-FIG. 6. Case 6, No. 6,383, age 24 years. Wounded Apr. 17, 1918. Severely wounded in shoulder and neck. Hemorrhage from wound of neck before admission to Casualty Clearing Station. Admitted to No. 13 General Hospital Apr. 18. Repeated small hemorrhages. Transfused Apr. 29. Operation—ligation of transverse cervical artery and vein and subclavian vein for arteriovenous aneurism. Transfused after operation. Good recovery.

This chart is included in the series for the purpose of showing the discrepancy between the hemoglobin estimations on capillary and venous blood in the presence of lowered blood pressure. When the first observations were made—some hours after hemorrhage—the hemoglobin in blood from the ear (marked *E* in the chart) was 48 per cent while that in the venous blood was 40 per cent. With a rise in blood pressure following transfusion this discrepancy diminished and the two readings finally became the same when the pressure reached 110 mm.

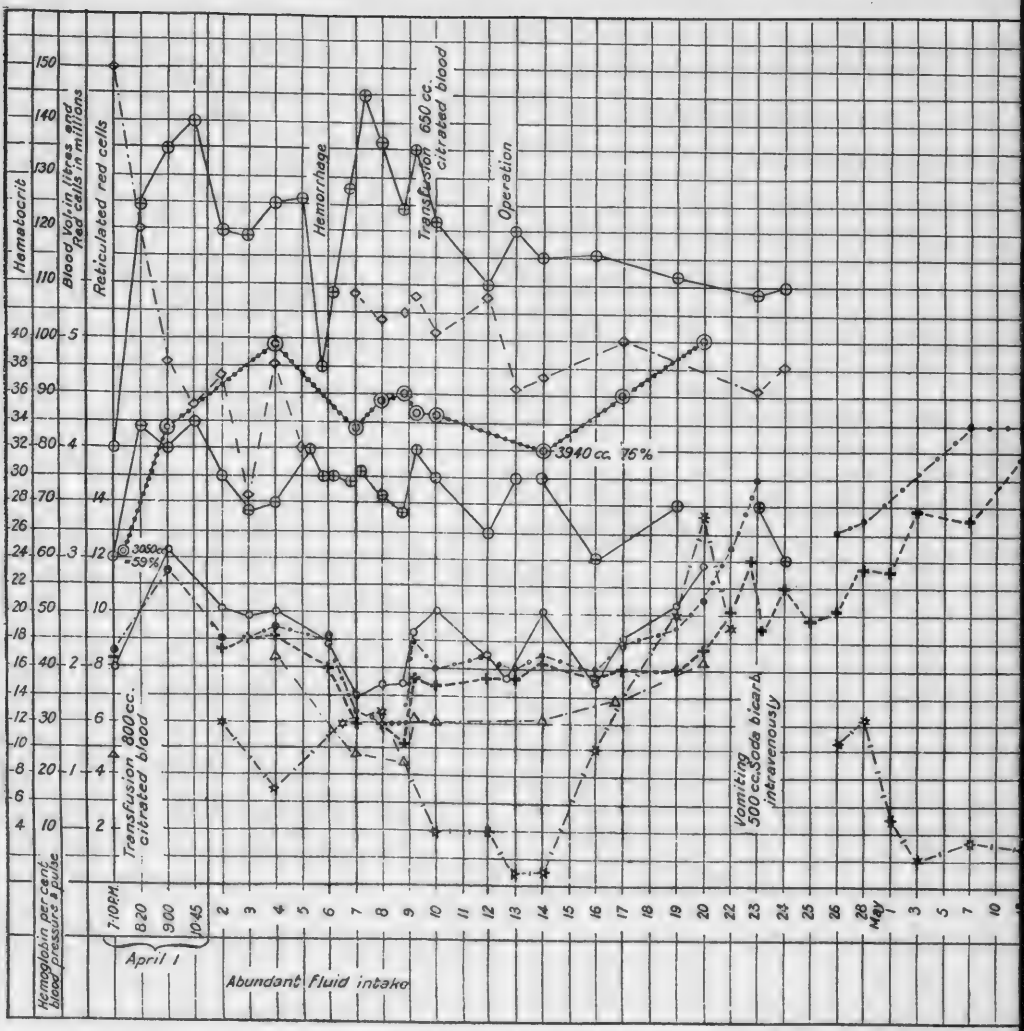
TEXT-FIG. 7. Case 7, No. 21,160, age 22 years. Wounded May 22, 1918. Multiple wounds, including compound comminuted fracture of fibula (left). At Casualty Clearing Station ligature of posterior tibial artery and vein (left). Admitted to No. 13 General Hospital May 30. No positive history of bleeding but evidence of previous hemorrhage in low blood pressure and blood findings. Treated by forced fluids. Good recovery.

This case is described fully in the text, page 159. The chart shows that the initial blood volume was only 62 per cent, but when treatment with forced fluids was instituted it rapidly increased to the normal. During this period of recovery there was marked fluid retention.

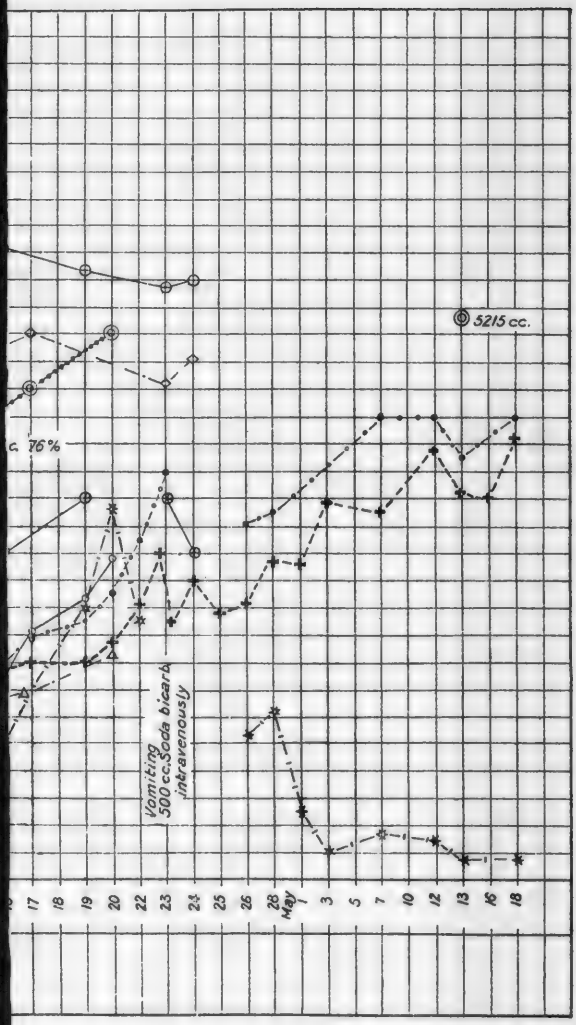
TEXT-FIG. 8. Case 8, No. 53,327, age 39 years. Wounded Apr. 29, 1918. Entrance wound below knee (right). Rifle bullet removed from groin. Fracture of lower third of femur. Moderate sepsis but wound apparently clean May 8. Uneventful course until May 12, when he had two hemorrhages. Femoral artery tied after transfusion of 450 cc. of blood and infusion of 800 cc. of saline solution. Died of gas bacillus infection May 14, 1918.

Immediately after transfusion the systolic blood pressure was 50 mm., diastolic 35 mm.; there was a well marked rise in pressure, after transfusion, to 120 mm. Observations on the blood began at this point. These are fully described on page 157.

TEXT-FIG. 9. Case 9, civilian, age 29 years. Wounded June 25, 1918. Partial amputation of both lower legs by train June 25. Observations begun 1 hour later. Operation approximately 3 hours after accident. Amputation of lower third of both thighs. During operation infusion of 550 cc. of gum acacia 5 per cent. Transferred to civilian hospital June 26. Good progress. This case is described fully in the text on page 161.




TEXT-FIG. 1. Case 1.


5215 cc.
c. 76%
Vomiting
500 cc. Soda bicarb
intravenously
17
18
19
20
22
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24
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May
1
3
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12
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16
18
Hemoglobin
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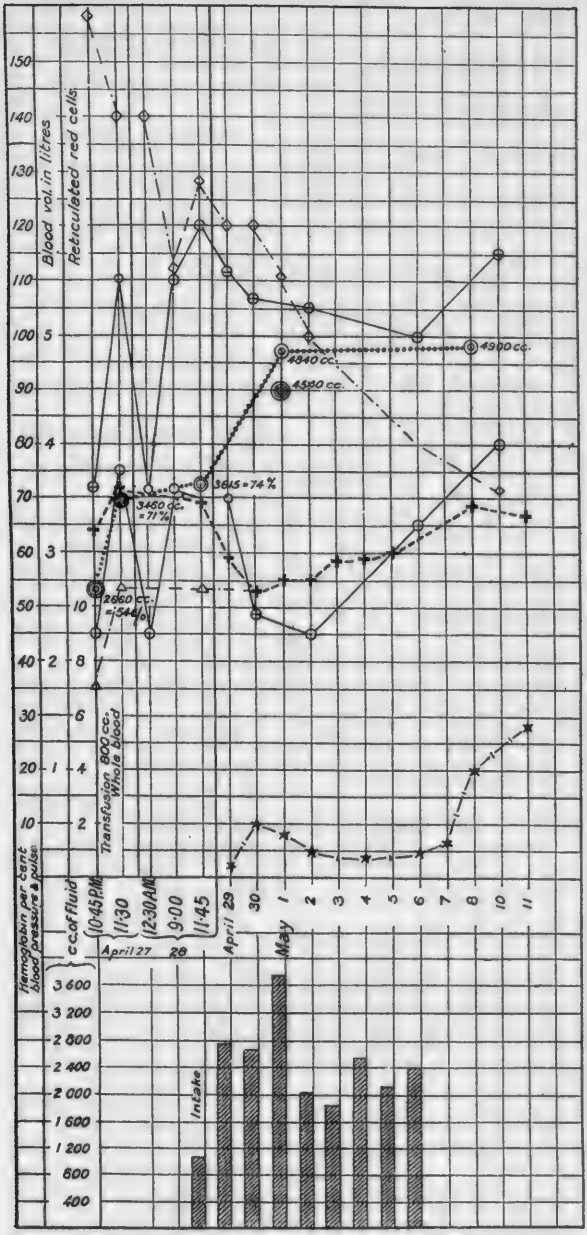
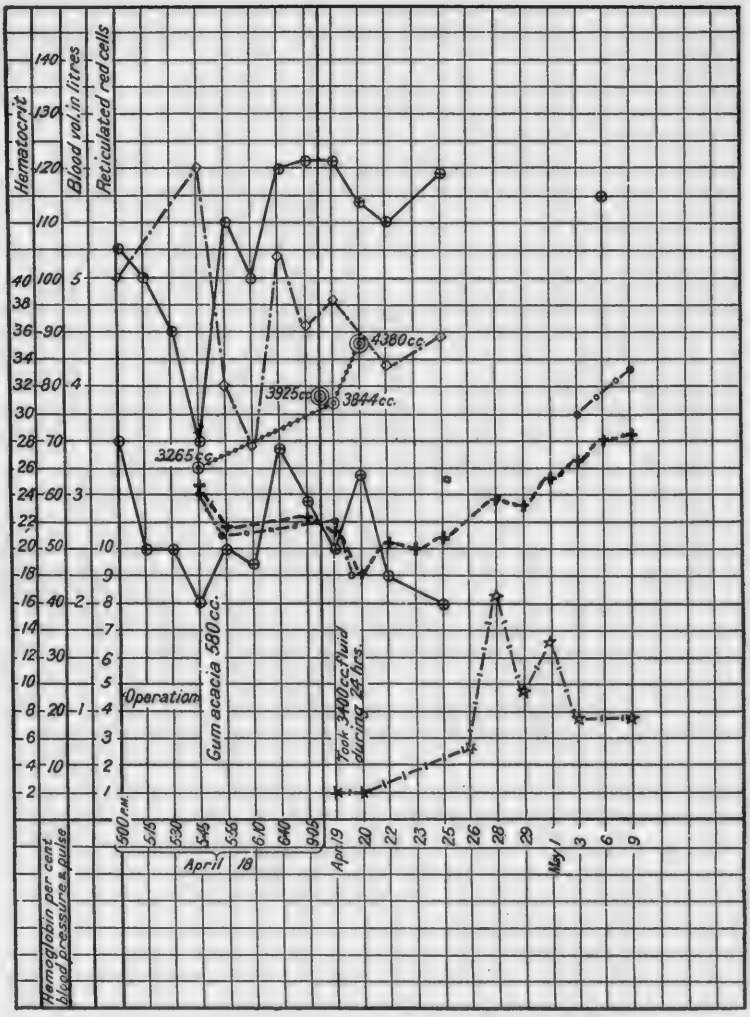
Red cell count
Reticulated red cells
oglobin change)
per cent

. Case 1.

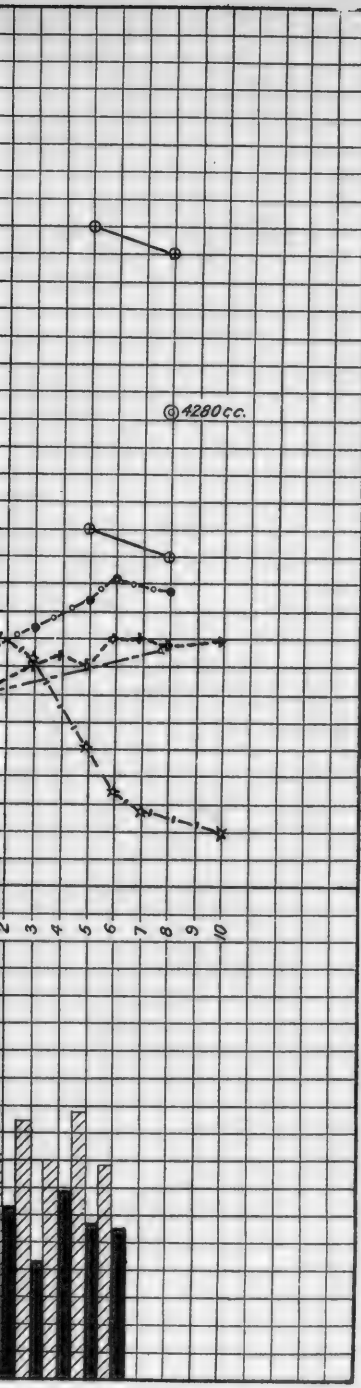



TEXT-FIG. 2. Case 2.




TEXT-FIG. 3. Case 3.

TEXT-FIG. 4. Case 4.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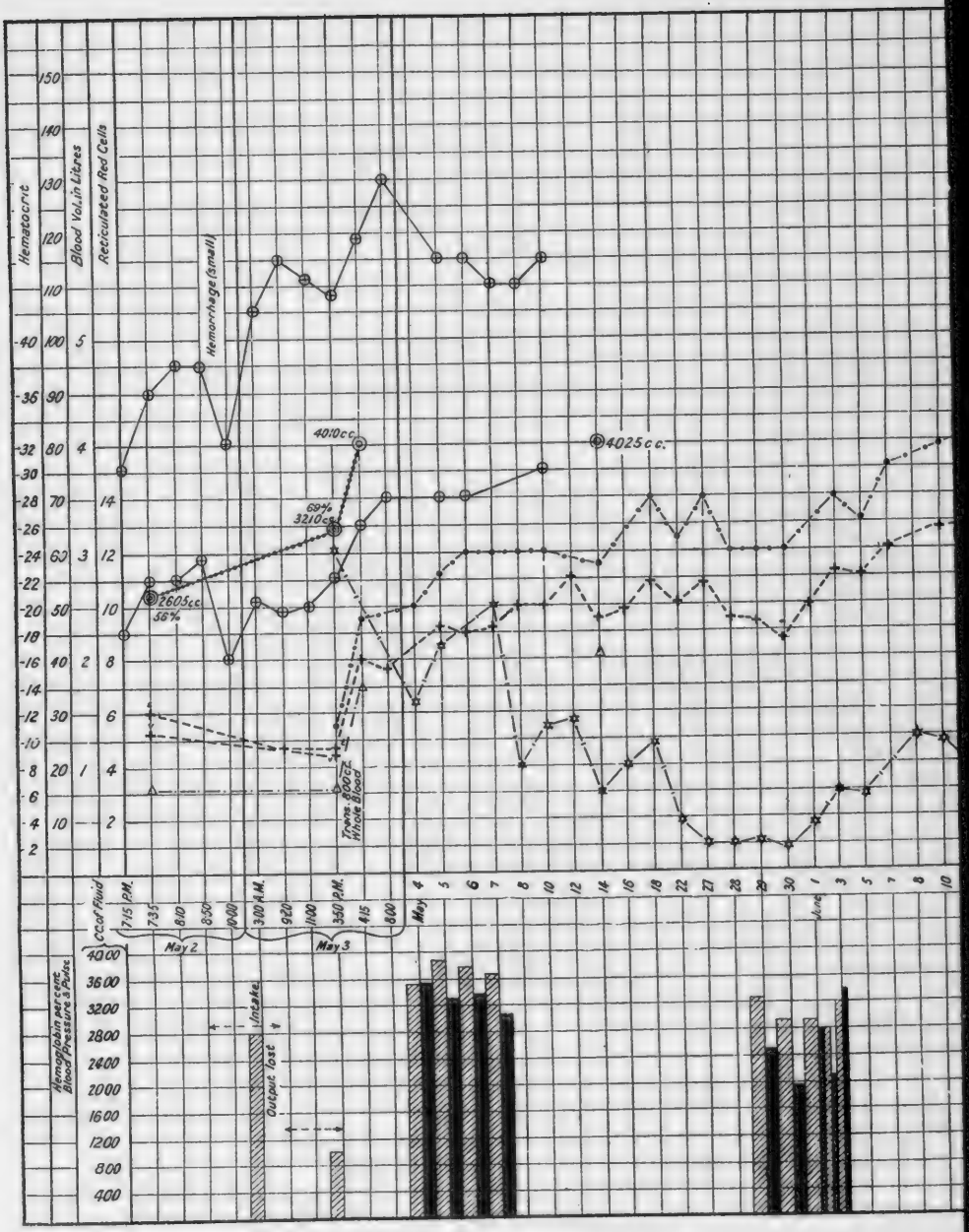
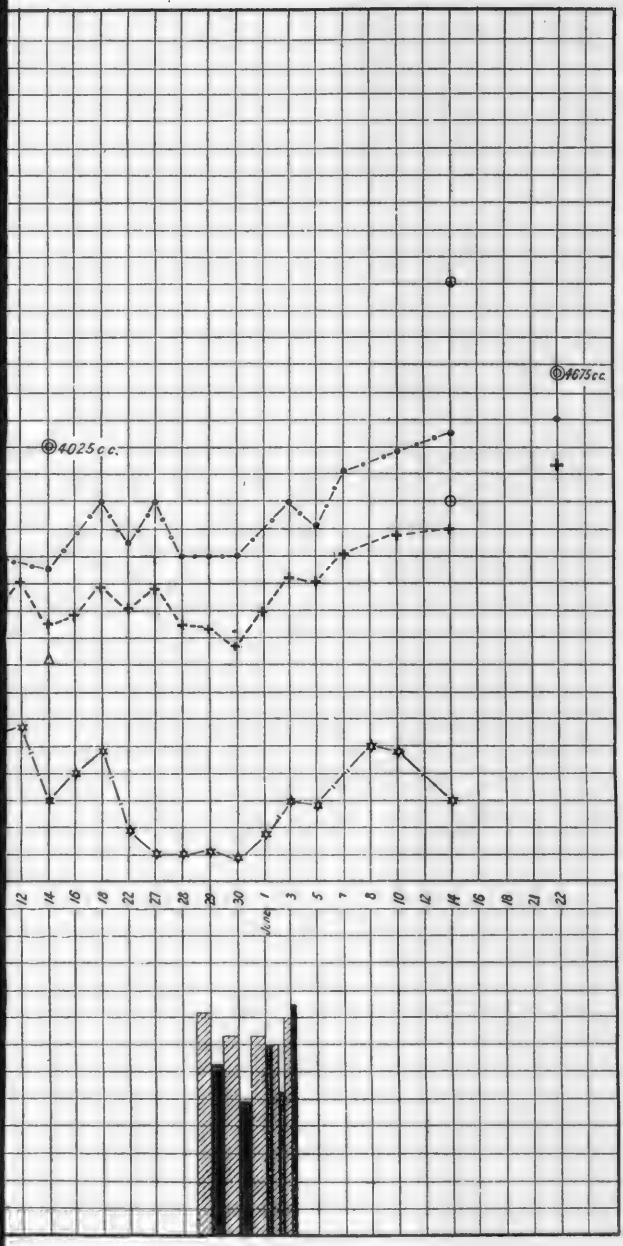
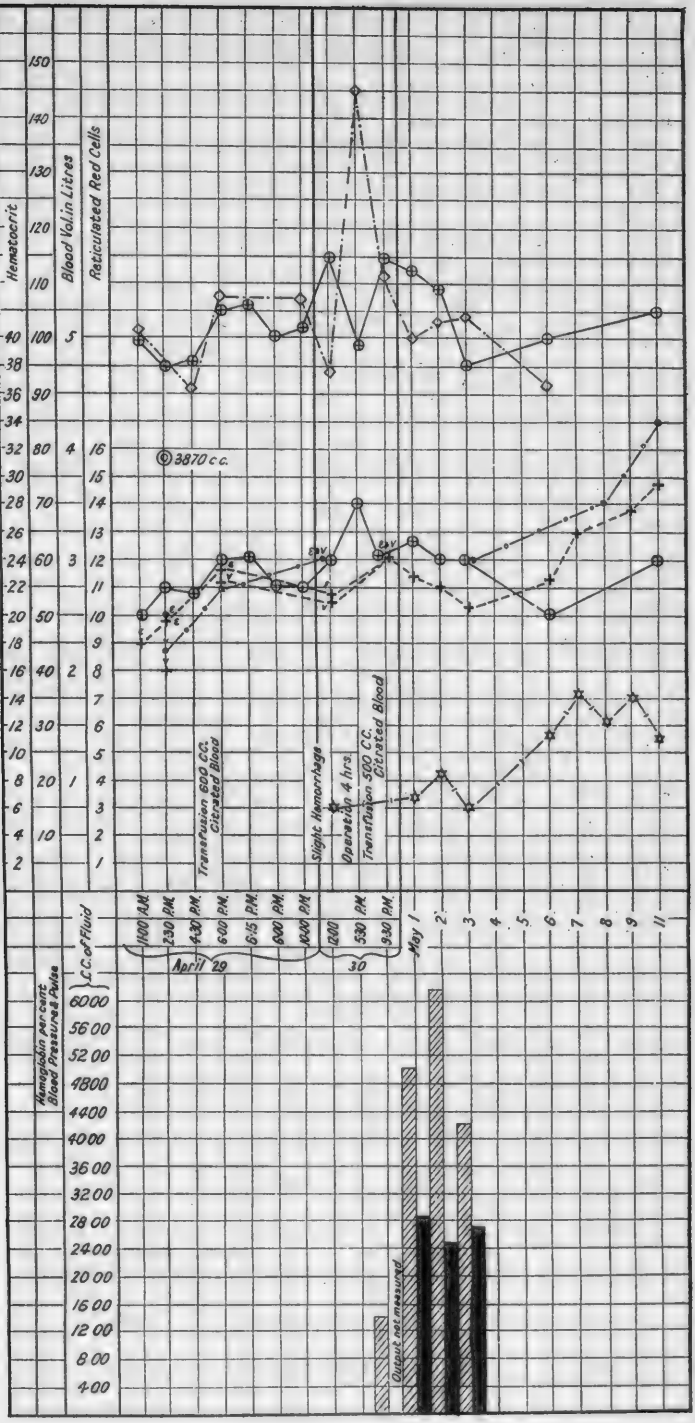


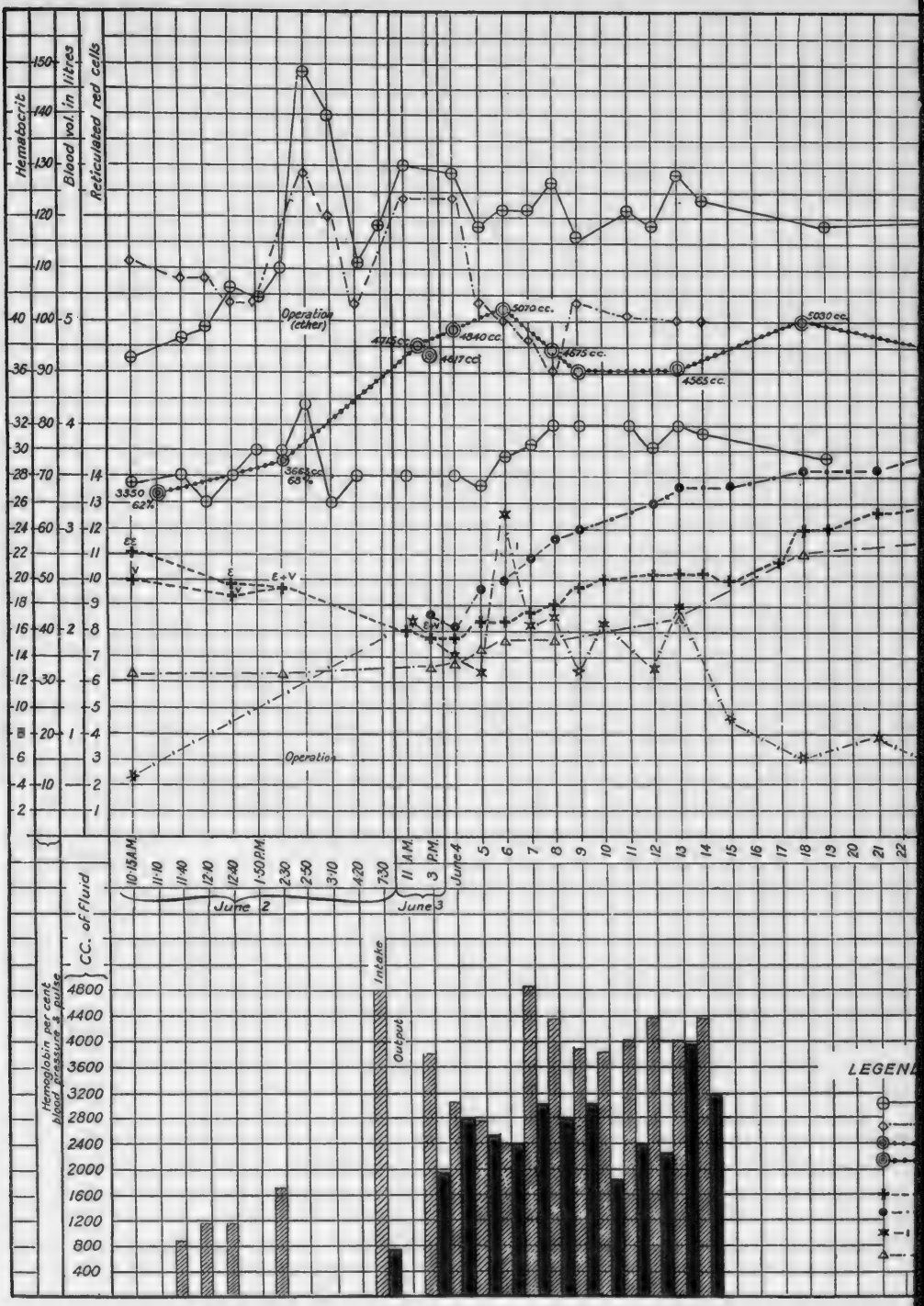

TEXT-FIG. 5. Case 5.



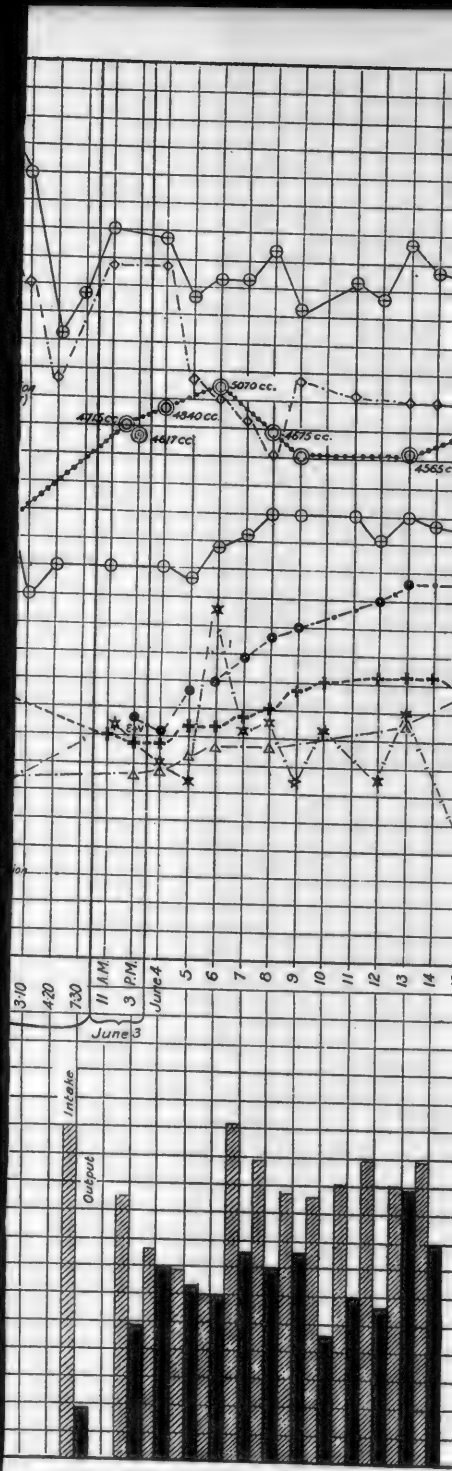

IG. 5. Case 5.



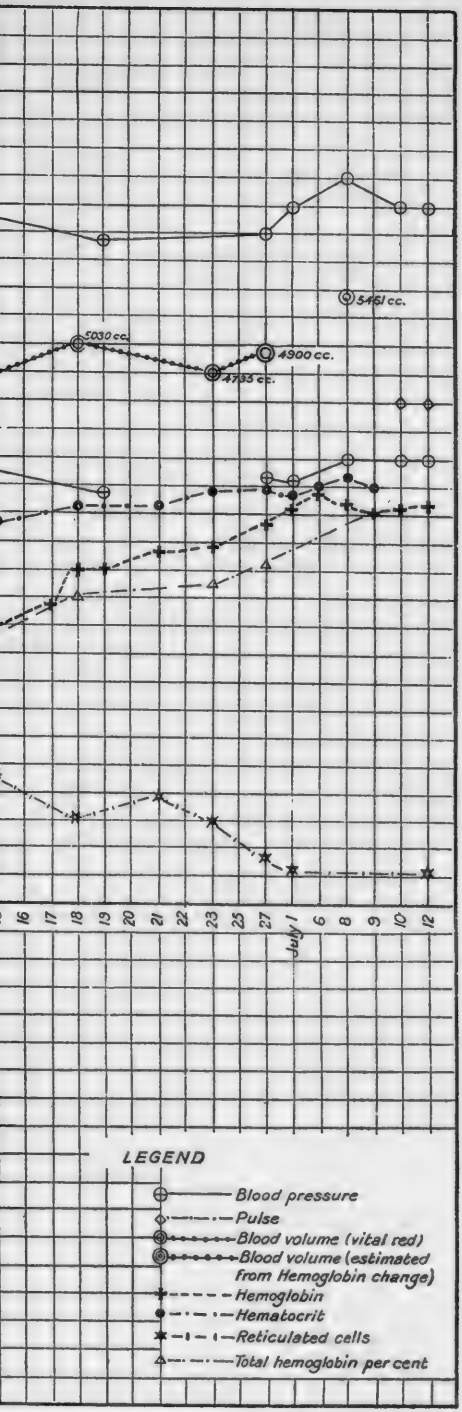

TEXT-FIG. 6. Case 6.




TEXT-FIG. 7. Case 7.




TEXT-FIG. 7. Case 7.


5461 cc.
5030 cc.
4900 cc.
4733 cc.
16
17
18
19
20
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22
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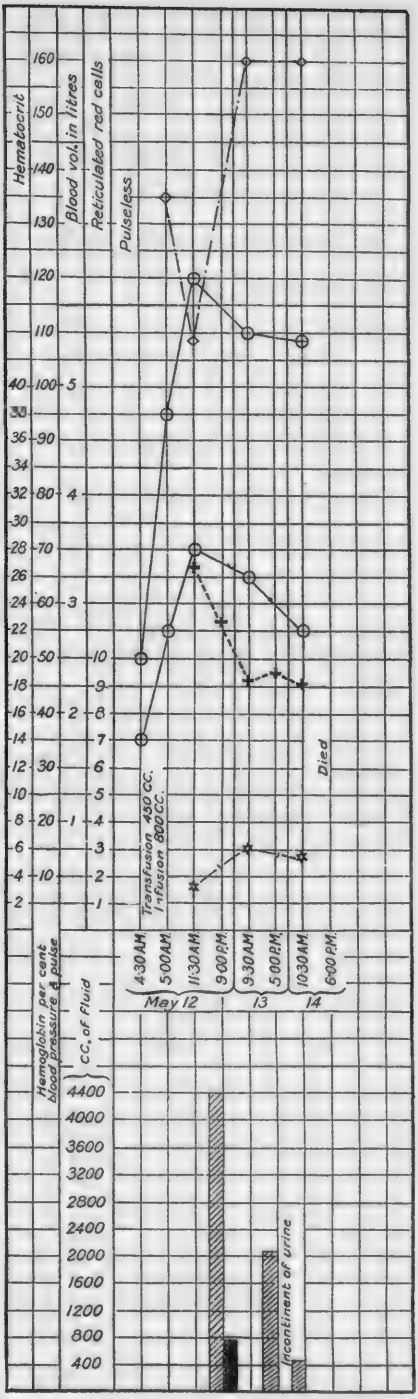
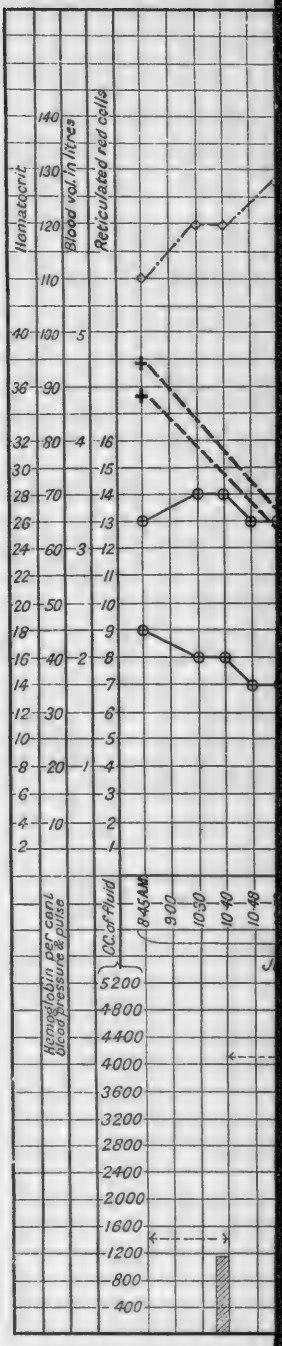
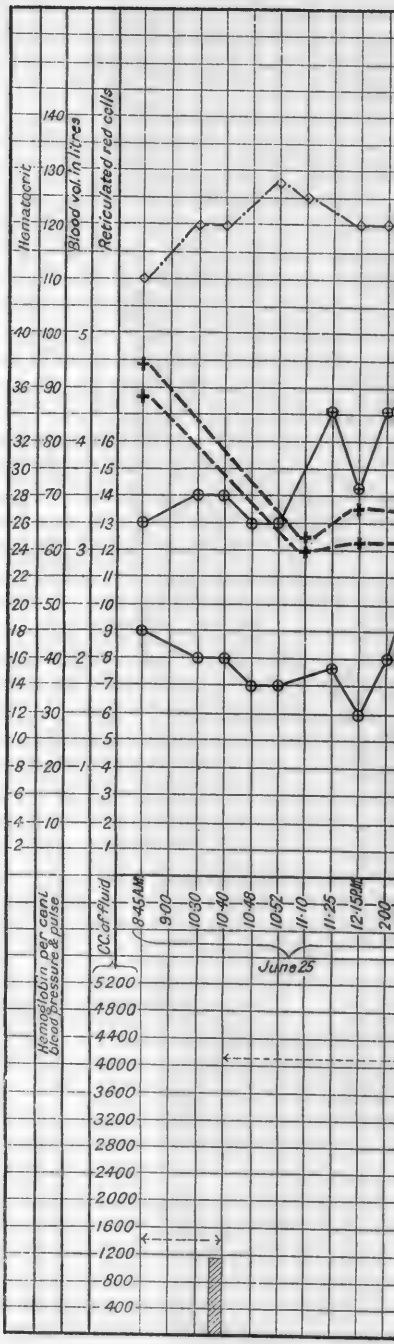
27
July 1
6
8
9
10
12
LEGEND
Blood pressure
Pulse
Blood volume (vital red)
Blood volume (estimated from Hemoglobin change)
Hemoglobin
Hematocrit
Reticulated cells
Total hemoglobin per cent



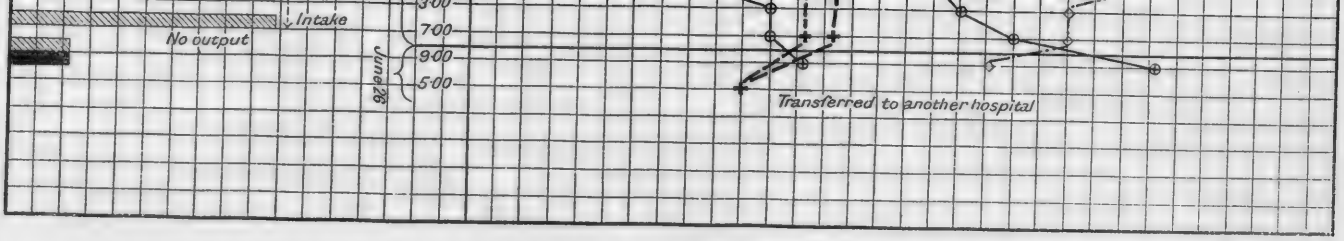

TEXT-FIG. 8. Case 8.




TEXT-




TEXT-FIG. 9. Case


Intake
No output
3·00
7·00
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June 26
Transferred to another hospital

# THE INFLUENCE OF ETHER ANESTHESIA, OF HEMORRHAGE, AND OF PLETHORA FROM TRANSFUSION ON THE PRESSOR EFFECT OF MINUTE QUANTITIES OF EPINEPHRINE.

By PEYTON ROUS, M.D., and GEORGE W. WILSON, M.D.

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(Received for publication, September 28, 1918.)

Epinephrine is still employed with more or less danger and success to raise the blood pressure in states of collapse. The fact lends some practical interest to the observations to be described here. Their original purpose was to determine whether the response of the blood pressure to the intravenous injection of very small amounts of epinephrine might not be a useful indicator of diminished blood volume in cases suspected of hemorrhage. In the course of the experiments ether was noted to have a marked effect on the response. And this point will be the first taken up, since it has a direct bearing on the other work.

## *General Method.*

Rabbits and dogs were used. Morphine and ether were the anesthetics given to the dogs, and paraldehyde, 1.5 cc. per kilo, was administered by stomach tube to the rabbits. A carotid artery was connected with a mercury manometer by the usual wash out cannula. The epinephrine injections were intravenous, into the external maxillary vein at its junction with the internal maxillary to form the external jugular. The flow in the jugular itself, from the internal maxillary, was utilized to flush the test doses into circulation, an important point in rabbits, since thus the introduction of a relatively considerable fluid bulk was avoided. The external maxillary ("posterior facial" in the rabbit) was dissected out for about 1.5 cm. above its junction with the internal maxillary ("anterior facial"), and a short, small cannula was introduced which carried on the end in the vessel a piece of narrow, soft rubber tubing about 0.8 cm. long. The end of the latter was pushed almost to the junction of the veins and held in place by a rubber-covered bulldog clamp, after which the sleeve of vessel wall was drawn up on the cannula and tied in place. When an injection was to be made, a hollow needle of exactly the length to reach to the end of the rubber tubing, and with a

rounded blunt tip, was thrust along the cannula, the bulldog clamp relaxed for an instant to permit its passage, and then closed on it again prior to the injection, which was practically instantaneous. In this way the test doses were delivered directly into circulation, and all injury to the vessel was prevented.

At the beginning of each experiment the minimum amount of epinephrine (Parke, Davis and Company) was determined which would bring about a well defined, if brief, rise of blood pressure, such as might conceivably be recognized in man by a coming through of the pulse beat in an arm compressed to just above the systolic pressure of the individual, as previously determined. It was found that in rabbits of 1,500 to 2,250 gm. our specimens of epinephrine caused regularly a pressure rise of 10 to 15 mm. of mercury when 0.5 cc. of a 1:1,000,000 dilution (the commercial solution diluted 1,000 times) was injected intravenously. This will be referred to hereafter as the minimum stimulative dose. Half the amount yielded only a negligible rise in pressure. The findings were identical in ten animals. The minimum stimulative dose caused no change in heart rate or amplitude, as indicated on rapid tracings, a fortunate circumstance, since thereby factors were ruled out which might have complicated the results.

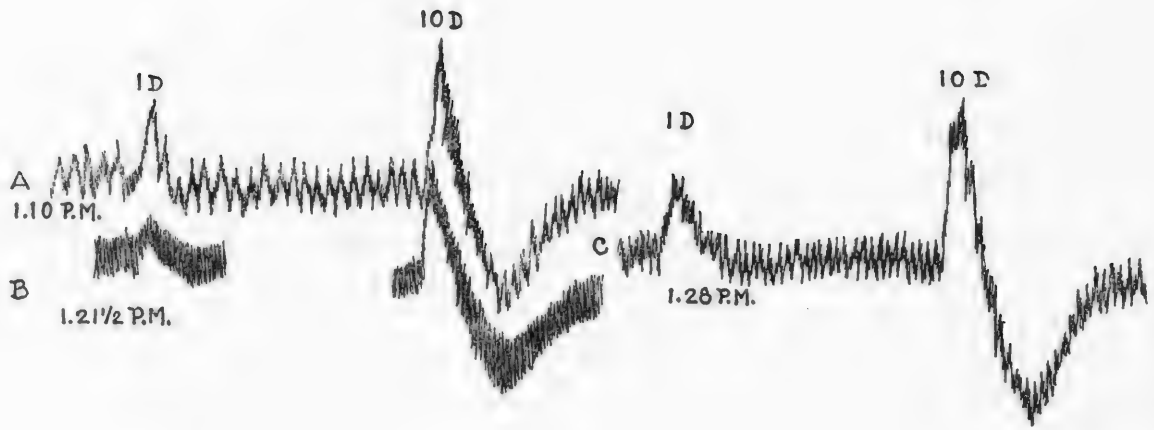
No attempt was made to determine whether the minimum stimulative dose was as constant in dogs as in rabbits.

At the beginning of each set of observations, the normal pressure response of the animal to 1 minimum stimulative dose of epinephrine and to multiples of it was several times recorded on a kymograph. The injections were given in a fixed order and at fixed intervals, and this sequence was followed throughout the experiment, in order that a possible influence of one injection to affect the response to the next might be disposed of as a disturbing element. As a matter of fact, 10 minimum stimulative doses did not suffice to alter the response to a like amount given 2 minutes later. But with 100 minimum stimulative doses it was necessary to wait more than 5 minutes if an identical response was to be obtained from a second injection. Consequently such large doses were seldom employed.

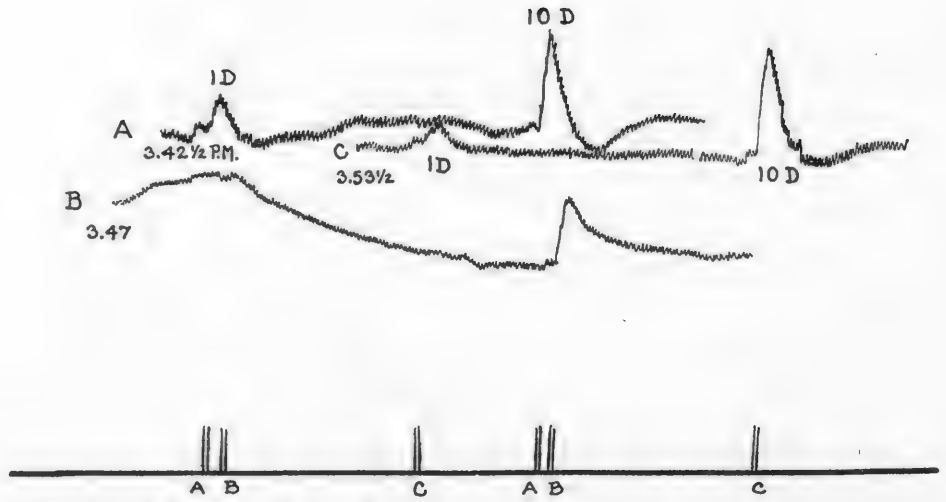
Save in special instances the fluid bulk injected at each test was 0.5 cc. Epinephrine in a dilution of 1:1,000,000 in 0.9 per cent sodium chloride loses its pressor effect entirely in 3 to 4 hours at room temperature, and for this reason fresh dilutions had to be made in long experiments. Fortunately the 1:100,000 dilution deteriorates little, if at all, in this time. By means of tests with this and with new 1:1,000,000 solutions, a constant check was kept upon the results.

### *Effect of Ether.*

In rabbits under the influence of paraldehyde, and in morphinized dogs, the amount of epinephrine required to cause a transient rise in the blood pressure of 10 to 15 mm. of mercury, that is to say 1 minimum stimulative dose, was found not to vary in successive tests on the




TEXT-FIG. 1. The effect of ether on the rise of blood pressure caused by epinephrine in a morphinized dog. 1 D, 10 D, 1 minimum stimulative dose, 10 minimum stimulative doses. The fluid bulk injected at each test was 1 cc. The base-line is the same for all the records. At *A*, when the blood pressure was normal, the animal was out of ether; at *B*, 11½ minutes later, deep ether anesthesia had been produced; while at *C*, the animal was out of ether again. The dog weighed 4,750 gm. and had received 22 mg. of morphine sulfate subcutaneously about 4½ hours prior to the first observation here recorded.




TEXT-FIG. 2. The effect of ether on the response to epinephrine in a rabbit under the influence of paraldehyde. *A*, prior to ether; *B*, deep ether anesthesia; *C*, out of ether. The animal weighed 2,250 gm. and was given 2.8 cc. of paraldehyde by stomach tube 2½ hours before the first test here shown.

same animal within a period of 2 hours. When ether was given alone, or with the anesthetics already mentioned, the pressor response varied with the amount employed. Under light ether the response was similar to that with paraldehyde, but when the ether was pushed to the degree sufficient for operative procedures, 1 minimum stimulative dose of epinephrine aroused almost no response, and even that to 10 minimum stimulative doses was somewhat diminished (Text-fig. 1). When still more ether was given, so that the blood pressure fell 20 to 30 mm., the rise caused by 10 minimum stimulative doses was far less than the normal for the same animal, and, in rabbits at least, it was slow in appearing and prolonged—which doubtless accounts in part for the lessened amplitude (Text-fig. 2). The response to large doses was never affected to nearly the same degree as that to small ones.

In the deeply etherized animals there was often some cyanosis. But cyanosis alone, as brought about by a gradual stenosis of the trachea, did not affect the epinephrine response.

### *Effect of Hemorrhage.*

There is much evidence to show that the blood pressure response to small doses of epinephrine is largely conditional on the state of contraction of the vascular muscle at the moment.[1] The cells are contracted more than usual, thus narrowing the stream bed, when the blood volume is diminished after a hemorrhage. These considerations led us to test the response in bled animals with a view to its possible clinical application. For there exists at present great need of some rapid indicator of lessened blood volume. According to Cannon and his coworkers,[2] and more especially to Govaerts,[3] an approximately normal blood count may be yielded by exsanguinated soldiers seen a few hours after their wound; and it may be impossible to distinguish clinically the collapse of lessened blood volume from that due to shock alone or an extending infection.[3]

[1] Cannon, W. B., and Lyman, H., *Am. J. Physiol.*, 1912–13, xxxi, 376.

[2] Cannon, W. B., Fraser, J., and Hooper, A. N., *J. Am. Med. Assn.*, 1918, lxx, 526.

[3] Govaerts, P., *Ambulance Ocean*, 1917, i, 355.

The extensive literature upon epinephrine contains almost no work concerning the effect of small amounts of the substance on a blood pressure lowered by hemorrhage. Hoskins, Rowley, and Rosser[4] bled dogs moderately (10 to 20 cc. per kilo of body weight) and injected an equal bulk of salt solution prior to tests with epinephrine. The blood pressure was not lowered by the bleeding and injection, and in view of the fact that no preliminary steps had been taken to deprive the animal's body of fluid reserves capable of making up the blood loss, it seems probable that only slight if any adjustment on the part of the vasomotor system could have been necessary. The authors conclude that "hemorrhage causes . . . . slight or no augmentation of the reaction to epinephrine."

In our experiments a reduction of the fluid reserves prior to the bleedings was deemed a primary consideration. Accordingly, both food and water were withheld from the animals during the 24 to 48 hours prior to operation. Relatively small bleedings in animals so treated brought about an enduring reduction of the blood pressure, whereas in those well watered this tended to return promptly to the normal after even considerable blood losses, and the effect of the epinephrine could not certainly be judged. The bleedings were accomplished through the free limb of the carotid cannula, which latter was disconnected from the manometer temporarily and flushed of anticoagulant. In reconnecting the manometer care was taken to lower its pressure beyond that which it was thought would now exist in the circulation. Thus any considerable entrance into the blood stream of the anticoagulant—half saturated sodium sulfate—was prevented.

A marked depletion was brought about by two to four successive bleedings. After each the response to epinephrine was several times tested. Then the blood bulk was restored, sometimes with horse serum or a 7 per cent solution of gum acacia in 0.9 per cent sodium chloride, but usually by returning the animal's own warmed blood, which had been kept from clotting by 0.5 per cent of sodium citrate. When the bleedings and reinjection were carried out rapidly, the blood pressure was usually restored to its original level. But when the animal had been kept in a severely depleted condition for 20 to 30 minutes, this result was not obtained, and even when fluid was injected in excess, the pressure remained low.

Paraldehyde and morphine were the anesthetics used, and in the manner already described. To control the results, ether was sometimes employed, always under careful restriction.

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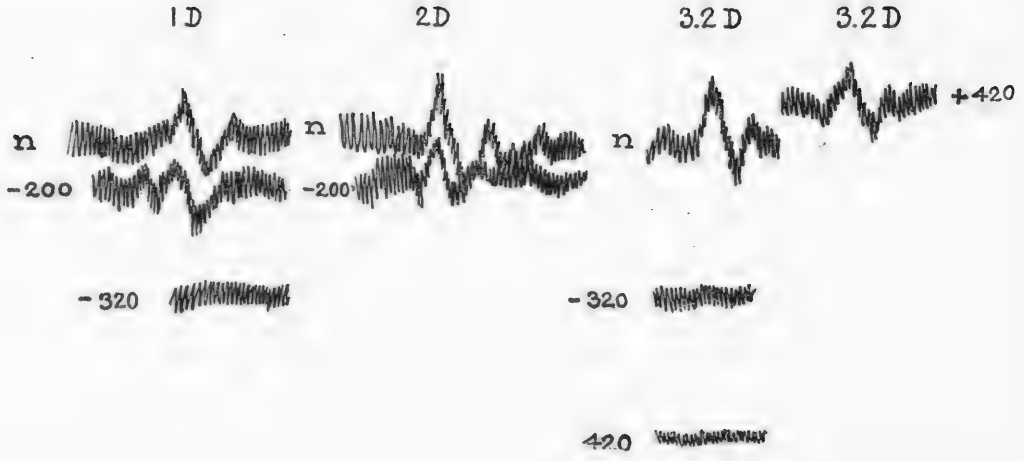
[4] Hoskins, R. G., Rowley, W. N., and Rosser, C., *Arch. Int. Med.*, 1915, xvi, 456.




TEXT-FIG. 3. The effect of hemorrhage and transfusion on the response to epinephrine of a morphinized dog. *n*, prior to bleeding; − 200, − 320, − 420, after the withdrawal of 200, 320, and 420 cc. of blood (in all); + 420 cc., after the reintroduction of the total blood removed. 1 D, 3.2 D, 1 minimum stimulative dose, 3.2 minimum stimulative doses respectively. The dog, weighing 11 kilos, received 66 mg. of morphine sulfate approximately 2½ hours before the first of the tests here shown.

The response to small doses of epinephrine was markedly affected by hemorrhages lowering blood pressure. Always a diminished pressure rise was noted, and the amount of the diminution was directly proportional to the lowering of the pressure consequent on the blood loss. When the pressure was very low, no response whatever was obtained from 1 or 2 minimum stimulative doses of epinephrine (Text-fig. 3), while sometimes 4 doses failed of effect. A response to 10 doses (0.5 cc. of a 1: 100,000 dilution) was never completely lacking, even in exsanguinated animals, but that seen was often sluggish and prolonged, as in animals heavily anesthetized with ether. That these results did not depend on mechanical inability of the cardiovascular system to raise the blood pressure, but to insufficient stimulus, was shown by the response to 100 minimum stimulative doses (0.5 cc. of a 1: 10,000 dilution), which was nearly always identical with the normal. Yet occasionally even this response was affected (Text-fig. 4).

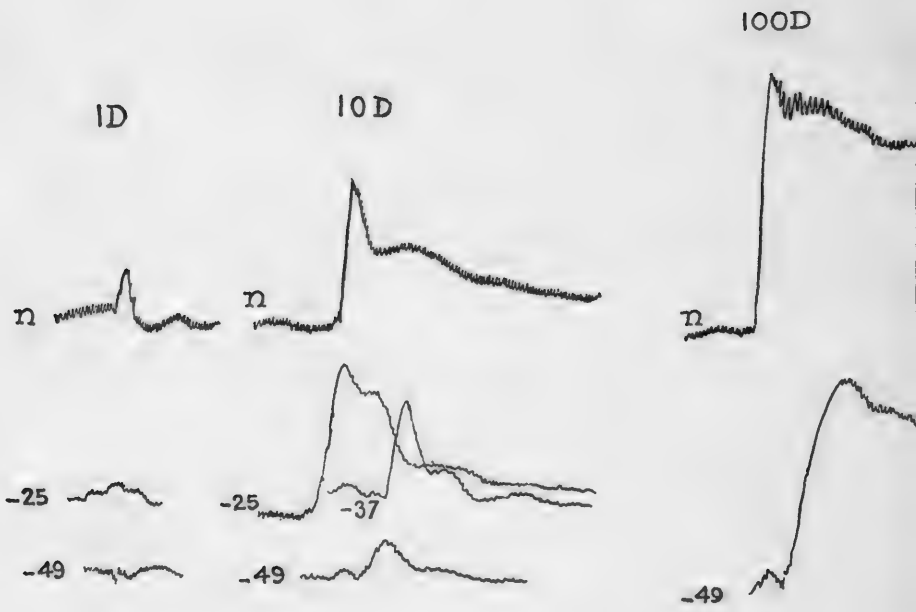
When the bleeding, testing, and reinjection of blood, or a substitute, were carried out briskly so that the blood pressure was low for only a few minutes, the response to epinephrine was not permanently impaired, but returned in proportion to the degree of restoration of the blood pressure. When the latter had reached the previous normal, the response was found to be approximately normal as well (Text-fig. 3). Prolonged depletion—for 20 minutes or more—had a lasting effect on the blood pressure which now could not be brought to the normal even by an excess of injected fluid. In such animals the response to epinephrine remained somewhat impaired (Text-fig. 4).

The results were the same in animals restored with horse serum or acacia solution instead of blood.

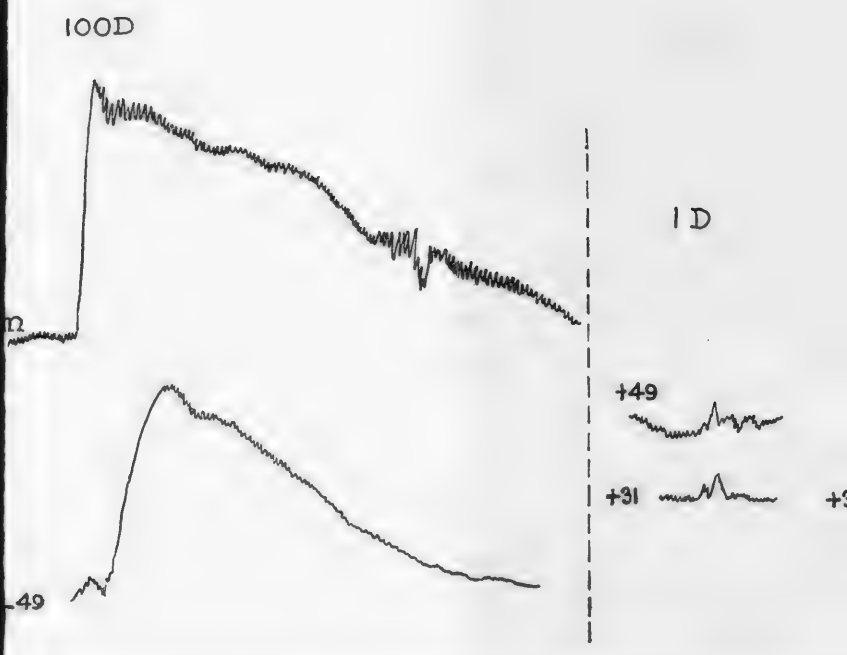
### *Effect of Plethora from Transfusion.*

As a corollary to the findings under the condition of diminished blood bulk the influence of plethora was tested.

Rabbits were used and were given paraldehyde as in the previous experiments. A few observations were also made in animals given ether, but these were not satisfactory, owing to the great alterations in the state of anesthesia which were produced by the fluid injections. As a rule the fluid used, which was always




TEXT-FIG. 4. The effect of hemorrhage and transfusion on the response to epinephrine of a rabbit wh
49, after the reinjection of 31 and 49 cc. respectively of the citrated blood. The chart shows the damagi
hours before the first of the tests here recorded.



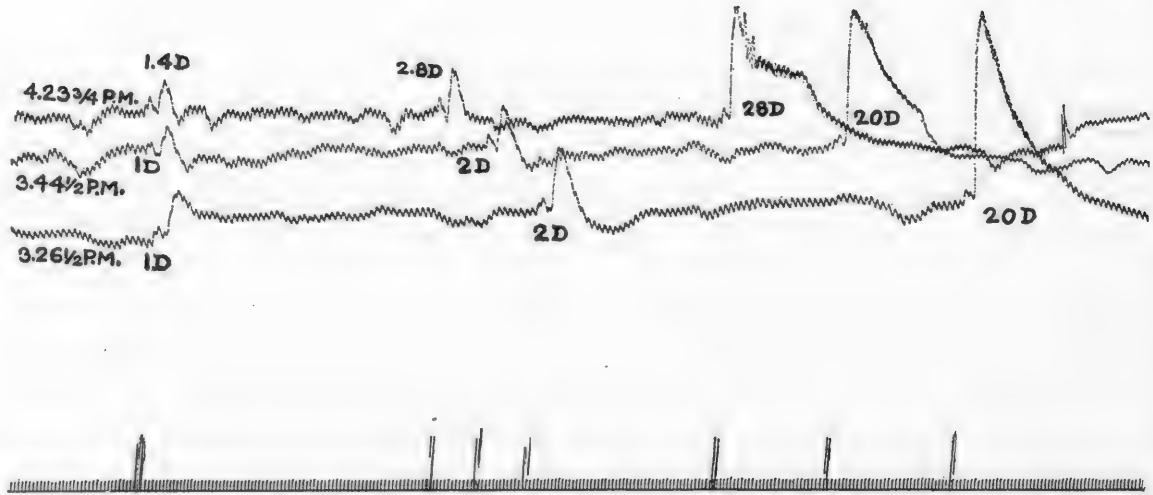

pinephrine of a rabbit which had received paraldehyde. *n*, prior to bleeding; − 25, − 3
chart shows the damaging effect on blood pressure of 20 minutes exsanguination. The r

, − 49, after the removal of 25, 37, and 49 cc. of blood (in all); + 31, +
bbit weighed 1,550 gm. and was given 1¼ cc. of paraldehyde approximately 3

warmed to 38–40°C., consisted of the citrated whole blood of other compatible rabbits; but sometimes a 7 per cent acacia solution was employed. Each time that the bulk of circulating fluid was increased it was necessary, of course, to increase proportionally the test doses of epinephrine in order to obtain comparable concentrations in the blood stream. For the calculations that this entailed, the original content of blood was assumed to be 5.5 cc. for every 100 gm. of the animal's crude body weight.[5] With each increase in circulating fluid produced by transfusion, the test dose of epinephrine was increased correspondingly; and it was diminished again as the fluid was withdrawn by bleeding.

The increases in blood bulk did not affect the blood pressure of the animals that were anesthetized with ether, but in those under the influence of paraldehyde every considerable increment of fluid caused a pressure rise which was permanent during the short period of the experiment. For example, an initial increase in the blood volume of about one-eleventh was usually attended by a pressure rise of 10 to 15 mm. of mercury. The observations were made then upon animals which were not only plethoric but had an abnormally high blood pressure as well. The response to epinephrine under such circumstances was regularly a diminished one; and the degree of the diminution varied directly with the heightening of the blood pressure. A diminished response was obtained even when the test dose was far greater than that necessitated by the increase in blood bulk (Text-fig. 5). When the excess of fluid was withdrawn by bleeding, the response again assumed normal proportions. The findings in plethora can scarcely be due to the sodium citrate delivered with the transfused blood, for the same results were obtained with acacia solution, and exactly opposite ones were obtained when the animals were bled prior to transfusion. However, to settle the point, the influence of citrate by itself was tested. The amounts employed in the transfusions were found not to affect the epinephrine response.

[5] Boycott, A. E., and Douglas, C. G., *J. Path. and Bacteriol.*, 1909, xiii, 256. Frequent tests of the rabbit's blood volume with vital red by a method modified from that of Keith, Rowntree, and Geraghty (Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, xvi, 547) have convinced us that this proportion closely approximates the real one.




TEXT-FIG. 5. The effect of high blood pressure with plethora from transfusion on the response to epinephrine in a rabbit. As in the preceding charts all the superimposed tracings are referable to the same base-line. The lowest tracing shows the results of tests under normal conditions; the next above the results of similar tests after the injection of 8 cc. of a 7 per cent solution of gum acacia in 0.9 per cent sodium chloride; and the highest the findings after a further 12 cc. of acacia solution. The acacia injections increased the blood volume by only $\frac{1}{11}$ and $\frac{3}{22}$ respectively of its original amount as calculated (5.5 per cent of the animal's crude body weight), yet they brought about notable rises in the blood pressure. For the tests in the normal condition and after the first acacia injection the same amounts of epinephrine were used; namely, 1, 2, and 20 minimum stimulative doses. After the second injection 1.4 times these amounts were employed, or nearly twice the increase that would have been proportional to the increase in blood bulk. The rabbit, weighing 1,600 gm., received 2 cc. of paraldehyde 3 hours prior to the first of the tests here shown.

## DISCUSSION.

It does not lie within our intent to discuss at length the physiological basis of the facts here recorded. The problems presented are obviously complex. But the findings in etherized animals cannot be passed over without a reference to the study of Auer and Meltzer[6] on the response of skeletal muscle as affected by general anesthesia with ether. These authors find that the muscle contractility is much diminished by ether, as shown both by direct stimulation and when the nerve is stimulated. They further conclude that the drug has a curare-like action, inducing a resistance to the passage of stimuli through the nerve endings. It seems probable from our work that some at least of these effects are exerted on smooth muscle. For the rise in blood pressure caused by minute doses of epinephrine is the result of contraction of the vascular smooth muscle, through stimulation at the myoneural junctions (Cushny). And when the animal is under ether the rise is much less or may be absent. One is not warranted in assuming without further work that this is the result of a diminished contractility of the vascular muscle, brought about perhaps by a block at the myoneural junctions, yet certainly it is the most likely explanation. And the effect of the ether is as transient as in the case of skeletal muscle. In proportion as the animal comes out of the anesthetic, the response to epinephrine returns.

The influence of hemorrhage on the pressure response to epinephrine may perhaps be due in part to a slowed or otherwise altered circulation of the substance in the depleted animal, so that an effective concentration fails to reach at a single moment the walls of those portions of the stream bed which are chiefly engaged in maintaining blood pressure. Whether such a factor is really active we are not enabled to say. But it will scarcely suffice to explain the lack of response to three or four times the test dose that is effective in the unbled animal. And in this connection it may be pointed out that the test dose undergoes far less dilution when injected into the circulation of an animal after the blood bulk has been decreased by severe bleeding than when injected prior to the blood loss.

[6] Auer, J., and Meltzer, S. J., *J. Pharmacol. and Exp. Therap.*, 1913–14, v, 521.

The character of the response to epinephrine when the blood pressure is lowered but the blood bulk remains intact varies with the means used to bring about the condition. If it is consequent on peptone shock or anaphylaxis, a lessened pressor response is obtained[7] for reasons as yet unknown. If, on the other hand, it has been induced by pithing or by the administration of nitroglycerol, both of which cause a relaxation of the vascular muscle, an increased pressor effect, or the substitution of one for a depressor effect, may be the result.[1] Here apparently the response is directly related to the state of the vascular muscle. Whether the lessened pressor effect observed in animals with a blood bulk decreased by hemorrhage is dependent on the unusually contracted condition of the vascular muscle—as was our *a priori* assumption—cannot be decided from the present experiments. But certainly if this is the case one is forced to conclude that only when the contraction of the muscle is very marked does any noteworthy effect obtain. For moderate bleedings which do not cause a lowering of the blood pressure, though they undoubtedly must result in some vascular contraction for its maintenance, have slight or no influence on the response to epinephrine.

The findings in plethoric animals have an interest in this connection. The repeated small transfusions by which plethora was produced would supposedly tend at some period to cause a relaxation of the vessels in order to accommodate the increased blood bulk. With such relaxed vessels an increased response to epinephrine might be expected. But as a matter of fact, any changes observed were in the direction of a lessening of the response. The marked lessening noted when the plethora was great and the blood pressure high is a new illustration of the oft noted fact that epinephrine may have a relatively slight influence on a blood pressure already above the normal (Cushny).

Neither ether anesthesia nor hemorrhage appreciably affects the pressure response to large doses of epinephrine. Herein doubtless lies the explanation for the fact that the marked influence of these factors on the response to small amounts has been overlooked. Yet this influence, as now demonstrated, is not without practical impor-

[7] Simonds, J. P., *Arch. Int. Med.*, 1916, xviii, 848.

tance. If epinephrine is to be administered to hemorrhage cases or to patients in collapse under ether, it should be given in the knowledge that doses effective under normal conditions may now fail to elicit a vascular response. If administered in considerable amount and intravenously, a temporary response, differing little if at all from the normal, can be obtained. But small amounts given subcutaneously with a view to causing a pressure rise within physiological limits will almost surely fail of effect.

It is probable that many factors besides ether anesthesia and hemorrhage will be found to lessen the pressure response to small doses of epinephrine. The statement has already been made that anaphylactic shock and peptone poisoning will do so. And we have noted that the pressure response gradually though slowly diminishes in animals given paraldehyde and kept on the operating table, with a manometer connection, for from 6 to 8 hours. From all this a successful clinical use of epinephrine as an indicator of diminished blood volume would seem unlikely.

## SUMMARY.

Ether anesthesia has a marked influence in diminishing the pressor response to minute amounts of epinephrine injected directly into the circulation. Hemorrhage also acts to lessen or abolish the response, and to a degree directly proportional to the lowering of the blood pressure it causes. In the exsanguinated animal an amount of epinephrine three or four times that sufficient to produce a pressure rise of 10 to 15 mm. of mercury under normal conditions, may be entirely without effect. The response to large doses, on the other hand, is uninfluenced by ether or hemorrhage.

The facts stated have a practical bearing not only on the employment of epinephrine to tide over collapse but on its possible utilization in the future to raise a low blood pressure to the normal height and maintain it during a considerable period. For the amount of epinephrine which under normal conditions will suffice to bring up the blood pressure may have little or no effect on an etherized individual or on one who has lost blood. The same difficulty will doubtless be encountered under other conditions.

In animals rendered plethoric by transfusion the response to small doses of epinephrine lessens in proportion as the blood pressure is increased by the plethora.

# BACILLUS EGENS. A NEW PATHOGENIC ANAEROBE.

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The bacillus described in this paper occurred in a fatal case of gas gangrene. The cultures were derived from infected muscle at a considerable distance from the wound surface, and no other anaerobes were present in the muscle. The bacillus proved markedly pathogenic for guinea pigs, producing extensive muscle lesions from which pure cultures of the bacillus were always obtained. Thus there is good proof that the bacillus found was the responsible agent in the fatal human case.

Morphologically the similarity of the bacillus to *Bacillus welchii* is marked. Its cultural reactions are such that in a mixed culture with *Bacillus welchii* it would escape notice, unless the most careful plating were done. When it has once been isolated, however, it is culturally and pathologically distinct.

*Case.*—X, age 20 years.

Day 1. Wounded. No Casualty Clearing Station note.

Day 2. Arrival at Base with multiple wounds of legs. There is a penetrating wound of the right thigh, and the whole thigh is much distended and tense. Clinically it is considered an obvious gas bacillus infection. X-ray examination shows two small foreign bodies, one in the right knee and one in the right thigh.

Day 3. A severe gas bacillus infection, extending up the abdominal wall. The thigh is blown up tightly, with gas crackles everywhere. Incision into upper thigh muscles reveals complete gas bacillus infection of the outer part of the thigh. Amputation is considered useless. 10 cc. of anti-gas bacillus serum are given intravenously in 200 cc. of saline solution, followed by 90 cc. undiluted.

Day 4. Condition is about the same, with slight increase in the extent of infection.

Day 5. 110 cc. of serum given intravenously. The gaseous emphysema now extends to the level of the costal margin, but the patient's general condition is not very bad. The thigh is still very foully gaseous. The patient vomits practically everything taken.

Day 7. Died at 1.15 a.m.

*Autopsy.*—Performed 9 hours after death. Extensive gas gangrene of the right thigh. The muscles are greatly swollen and gaseous, gangrenous in the oldest part of the lesion, showing the usual variations in hue according to the age of the lesion. There is marked friability of the muscles. There is a small amount of subcutaneous edema, and edema between muscles, with reddish fluid. Iliopsoas muscles within the abdomen pale and soft but not gaseous. *Kidneys.*—Slightly swollen and pale on section, with markings obscure. *Spleen.*—Small. Appears normal on section. *Liver.*—Acute degenerative changes. *Lungs.*—Normal. *Heart.*—Normal. *Peritoneum.*—Normal.

*Specimen Used for Cultures.*—A large piece of muscle was excised aseptically from the right thigh at operation, before treatment was started. None of the original wound surface was included in the specimen. On arrival at the laboratory the surface was seared and pieces were cut with a sterile knife from within the mass, planted in chopped meat medium, smeared on egg slants, and incubated anaerobically in McIntosh and Fildes' jars.

*Cultures.*—In 24 hours numbers of isolated colonies were present on the egg slant. All appeared alike. Several were fished into milk, but gave no reaction within 4 to 5 days. Slides of the colonies showed a bacillus resembling *Bacillus welchii*, varying more in length, however, than is usual with *Bacillus welchii* under similar conditions. Further incubation and observation of the egg slants showed no new developments. The milk test was repeated on numerous colonies, but was always negative.

The chopped meat culture gave a bacillus resembling *Bacillus welchii*, but heavily planted subcultures in milk gave no reaction in spite of good growth. Streaked on egg slants, it gave only colonies like those from the original muscle planted on egg.

Colonies from the original egg slant and from the meat subcultures were then plated on egg slants and single colonies were picked and immediately replated. The resulting colonies were always alike in gross and microscopically. After the plating had been repeated a number of times, several colonies were picked and run through media in parallel for determination of characteristics.

The above account of the cultural methods shows that *Bacillus welchii*, if present, could not have been missed, for it would have given the characteristic milk reaction in the plantings from the original

cultures. The milk used was tested by running a *Bacillus welchii* in parallel. It also proves that the original muscle contained a pure culture of the bacillus in question.

### *Characteristics of Bacillus egens.*

*Morphology.*—The bacillus is morphologically practically identical with *Bacillus welchii* when grown in most liquid media. It is of the same length, thickness, and shape. In animal tissues and in the first subcultures in bouillon the bacillus shows a well defined capsule, easily stained by gentian violet according to the Welch method. The bacillus is strongly Gram-positive in young cultures.

The differences in form between *Bacillus egens* and *Bacillus welchii* are evident in surface colonies of 2 days or more on a medium which is not alkaline. Occasionally *Bacillus welchii* produces long thread-like forms in old cultures on alkaline sugar-free media, but there is little variation in length on 0.5 per cent acid glucose agar or on the neutral egg slants. *Bacillus egens*, however, in cultures over 1 day old produces long thread-like forms on these media, often in great abundance. They are especially marked in the condensation water of egg slants and on glucose agar. These long forms are tortuous, unjointed, and Gram-positive. From forms five to ten times the length of *Bacillus welchii* they soon grow to a length extending from one to three times the diameter of an oil immersion field.

The second principal difference in appearance consists in the early appearance of Gram-negative forms of *Bacillus egens*. Often 50 per cent of the bacilli in surface colonies appear Gram-negative in 2 days. In cultures 4 to 5 days old nearly all the bacilli except some of the long threads are Gram-negative, and a great many of them are greatly degenerated, forming small, non-refractive, swollen, nearly spherical masses. *Bacillus welchii* shows few if any Gram-negative forms under corresponding conditions, and no similar degenerated forms. This tendency to Gram-negative forms is less evident in liquid media.

Another less definite difference consists in an unevenness of outline in 1 day milk cultures which *Bacillus welchii* does not show.

*Motility.*—The bacillus is absolutely non-motile in cultures, and in the infected fluids of animals. *Bacillus fallax* and *vibrion septique*

were used for a test of method, and both were found definitely motile in the medium used (condensation fluid of egg slants).

*Spores.*—No evidence was found of spore formation.

### *Cultural Characteristics.*

*Anaerobiosis.*—*Bacillus egens* is a fairly strict anaerobe. No growth could be obtained aerobically on slants, and an almost imperceptible cloud formed in the depths of a glucose bouillon tube which had been shaken with air.

*Proteolytic Action.*—No proteolytic action occurs on the casein of milk, coagulated egg albumin, cooked muscle, or coagulated horse serum, even after long exposure. This is in contrast to its softening effect on living muscle in infected animals. *Bacillus welchii* shows the same contrast in less degree.

*Saccharolytic Action.*—The only sugar fermented quickly and vigorously is glucose, in which marked acid and gas formation occurs in 24 hours. Some of the other sugars are feebly attacked. Often they show no change or a very slight one unless a heavy planting is made. Serum water media were used, and a culture of *Bacillus welchii* run as a control.

Maltose and saccharose under favorable conditions show slight acidity and gas in 24 to 48 hours. Lactose is fermented with great slowness and feebleness, never showing more than a trace of acidity to litmus. In milk not enough acid is produced to cause an acid clot in less than 12 to 14 days, even with a heavy growth.

Galactose and raffinose are fermented to the same degree as saccharose.

Mannite, dulcite, inulin, glycerol, and salicin are not changed.

*Indol Production.*—No indol is produced.

*Hemolytic Action.*—Surface and deep colonies on blood agar show a definite hemolytic zone in 24 hours.

*Odor.*—No noticeable odor was produced in any cultures.

### *Appearance of Cultures.*

*Milk.*—No gross change occurs in milk in less than 12 days, and usually not for 15 to 20 days, when a slow precipitation of casein

occurs followed by acid clot formation with little or no gas. The fluid above the clot is cloudy and gray. No digestion of the casein or further change occurs.

This absence of the stormy fermentation seen with *Bacillus welchii* is a constant and definite character of *Bacillus egens*, whether the cultures are freshly isolated from animals or have been under cultivation for 6 to 7 weeks. Tests were made with numerous cultures with heavy plantings, and good growth occurred within 24 hours. *Bacillus welchii*, run in parallel on the same medium, always produced the characteristic stormy clot.

*Chopped Meat.*—A few gas bubbles, but no other change. No digestion, blackening, or other color production.

*Egg Slants.*—No blackening or softening.

*Glucose Bouillon.*—Diffuse cloudiness with fine sediment. Diffuse cloud persists indefinitely.

*Plain Bouillon.*—Faint diffuse cloud.

### *Colonies.*

*Glucose Agar.*—In 24 hours the colonies are 0.5 to 2 mm., rather flat, circular, with a sharp even edge. By transmitted light against a black background they appear a bluish gray with scarcely greater opacity in the center. With a hand lens the colony is seen to have a very fine grayish grating in a clear translucent stratum. With the light at the proper angle reddish, purplish, and green iridescent tints are visible with a hand lens. They are probably due to refraction of the light by the fine internal structure of the colony. *Bacillus welchii* colonies under similar conditions have a large, dense, opaque, yellowish white central portion, with a translucent border filled with coarse white flecks, and show no iridescence. *Fallax* colonies are like *Bacillus welchii*, but a little less opaque and coarse.

Further incubation produces a slight increase in size and the refractive qualities decrease, but otherwise there is no special change.

Deep colonies on glucose agar are very small ($\frac{1}{3}$ mm.), opaque, white, lenticular specks without processes. They change little in size. *Bacillus welchii* produces much larger colonies and forms a larger amount of gas. On egg slants the colonies are circular with a

sharp regular outline. Within 24 hours they are pale gray but soon become a light yellow color. The surface is extremely smooth, even, and shiny. The size varies from 1 to 2 mm. at first, to 4 to 5 mm. in 2 to 3 days. *Bacillus welchii* in comparison shows after 2 to 3 days a grayish white, elevated center, with a flat, dirty yellow, slightly crenated border. Both have a surrounding zone of light discoloration of the egg medium.

Deep colonies in blood agar become brown in 2 to 3 days with a small, central, darker brown dot.

### *Pathogenic Action.*

*Bacillus egens* proved highly pathogenic for guinea pigs and somewhat less so for rabbits. After 6 weeks of subculture 0.1 cc. of a 24 hour bouillon culture was fatal to guinea pigs. After passage through guinea pigs 0.02 cc. of a 24 hour glucose bouillon culture was fatal within 30 hours. Experiments in rabbits were few, but 2 cc. of a 24 hour glucose bouillon culture was fatal to a rabbit in 2 days.

The disease in guinea pigs has the following characters.

*Clinical Characteristics.*—1. Constitutional symptoms of dullness, apathy, and sluggishness of reflexes. Fever is followed by subnormal temperature.

2. Local signs consisting in a marked soft fluctuant swelling at the site of inoculation with brownish or slightly greenish discoloration of the skin. There is local tenderness and warmth.

3. Soft sodden edema over abdomen, chest, and neck.

4. Slight gas crepitus over site of inoculation, abdomen, and chest.

*Pathological Characteristics.*—1. Extensive and complete muscle destruction in the region of inoculation. Many of the muscles are reduced to soft, pale, pulpy fragments lying in a thin red fluid. On picking up one of the pieces it falls apart by its own weight. Muscles less affected are easily torn. All the lesions are characterized by extreme pallor of the muscles. *Bacillus welchii* produces some local muscle destruction but not to such a degree, and the muscles have a different color.

2. Extensive muscle lesions distant from the point of inoculation, especially over the abdominal wall and chest. Sometimes the muscles

of the opposite leg are affected. The muscles are pale with a faint, light, purplish pink tinge, are very soft, and tear with great ease.

3. A pseudogelatinous dull red edema, usually marked in the flanks and axillæ, and often thick over the sides of the abdominal wall. It appears gelatinous to sight and touch, but on cutting it a thin, slightly brownish red fluid escapes and the immediate area entirely collapses. It therefore simply consists in a thin fluid held in very small compartments made up of thin translucent recent adhesions.

4. Gas bubbles thinly scattered in the subcutaneous tissue of abdomen and chest.

5. Absence of any odor different from that of a normal dead guinea pig.

6. No destruction of skin or loosening of hair.

7. Invasion of peritoneal cavity and blood stream.

A positive blood culture at death, or soon after in the cases where autopsy was not done immediately, was always obtained and usually bacilli could be found in blood films.

In the following protocols of experiments all these details will not be given as they are qualitatively the same in each case.

*Technique.*

Each animal was shaved at the site of inoculation and the skin cleaned with methylated spirits followed by ether and iodine. The cultures were injected into the muscles of the thigh with a very fine, sharp needle, so that trauma of muscle was reduced to a minimum as a factor in the pathogenicity. Unless otherwise stated the cultures were 24 hour growths in 1 per cent glucose bouillon.

*Guinea Pig 1.*—2 cc. of a 2 day culture in chopped meat medium (rather thin growth). In 24 hours marked local signs of the usual character. The guinea pig appeared dull and sick. Died 36 hours after inoculation.

*Autopsy.*—Performed ½ hour post mortem. A large pocket is found at the site of inoculation filled with thin red fluid in which lie pulpy muscle fragments. Nearly all the muscles of the thigh are gone. Subcutaneous edema over abdomen and chest with a few gas bubbles. *Peritoneal Cavity.*—No fluid.

*Cultures.*—Pure cultures from the edema fluid, affected muscle, and heart's blood.

*Films.*—Films from the edema fluid show large numbers of the bacilli, which are short and resemble *B. welchii*. There are practically no cells. Blood films show a few bacilli.

*Guinea Pig 2.*—The same inoculation as Guinea Pig 1. Similar clinical course and pathological condition.

*Guinea Pig 3.*—0.3 cc. of a 24 hour glucose bouillon culture from a colony from the heart's blood of Guinea Pig 1. Died in 18 hours.

*Autopsy.*—Performed within ¾ hour after death. Lesions similar to those of Guinea Pigs 1 and 2 with a smaller local pocket of fluid. Cultures and films give similar results, with the addition of a positive culture from the peritoneal cavity.

*Guinea Pig 4.*—0.1 cc. from the original culture. (No passage through animals; under cultivation for 6 weeks.) Died in 28 to 36 hours.

*Autopsy.*—Performed 1 to 8 hours post mortem. Lesions of the usual type. Pure cultures from edema, muscle, heart, and peritoneum.

*Guinea Pig 5.*—The same inoculation as Guinea Pig 4. In 12 hours marked local swelling, tenderness, and discoloration of skin. Guinea pig sluggish and sick. In 36 hours local swelling is slightly less, the animal appearing better. Recovery later, with persistence of a slight local swelling.

*Guinea Pig 6.*—0.01 cc. of the same culture as Guinea Pig 4. No evident effects.

*Guinea Pig 7.*—0.1 cc. of a culture from a colony from Guinea Pig 4. Died in 29 hours.

*Autopsy.*—Performed ½ hour post mortem. Usual lesions. Pure cultures from edema, peritoneum, and heart.

*Guinea Pig 8.*—0.02 cc. of the same culture as Guinea Pig 7. Died in 29 hours.

*Autopsy.*—Performed within 1 hour post mortem. The usual lesions. Pure cultures from edema, peritoneum, and heart.

*Rabbit 1.*—2⅛ cc. of a 2 day chopped meat culture (from culture not passed through animals; rather thin growth). Local swelling in 24 hours. The animal appears somewhat sluggish and ill. Killed with chloroform in 48 hours.

*Autopsy.*—A local abscess in the muscles of the thigh, caused by destruction of muscle tissue, well walled off. The muscles nearby, however, were pale, nearly translucent, crisp, and friable. Further off the muscles were pale and easily torn. Edema was present in the flanks as in the guinea pigs.

*Cultures.*—Pure growth of *B. egens* from the muscle.

*Rabbit 2.*—2 cc. of a culture recently isolated from a guinea pig. In 24 hours slight local swelling. The rabbit appeared sluggish. In 48 hours the local swelling had increased, the leg was apparently paralyzed, and the rabbit appeared extremely sluggish and dull and felt cold to the touch. There was slight crepitation over the local swelling and over the abdomen. Death occurred that night.

*Autopsy.*—At the site of inoculation the muscle was pale externally and on section, friable. The muscles were slightly swollen, and between them were

a dull red pseudogelatinous edema and gas bubbles. A similar edema and gas extended over the abdominal wall and chest. There was marked edema in the flanks and axillæ. The muscles of the chest and abdomen were pale, soft, and friable. *Peritoneal Cavity.*—A small amount of slightly cloudy, pale fluid, containing numerous bacilli.

*Cultures.*—Pure cultures from muscle, edema, peritoneum, and heart.

### *Pathology of the Lesions.*

Sections of muscle from the human case showed muscle degeneration much as in *Bacillus welchii* infection, but without any hemorrhages. There was practically no cellular infiltration. Bacilli were not numerous except in a few areas. No other organisms could be found.

*Kidney.*—Sections showed marked acute tubular degeneration. The nuclei were pyknotic or had disappeared; the protoplasm was swollen and granular. No bacilli were found.

*Liver.*—Fairly well marked degeneration of the central type. No foci of infection.

*Guinea Pig Muscle.*—The infected muscle shows marked edema between muscle fibers, practically no cellular infiltration, and no hemorrhage. The muscle fibers degenerate either in mass, losing structural markings and becoming deep staining, or, and this very frequently, becoming edematous, with separation of the fibrillæ, which still appear sharp and distinct. Bacilli are very numerous between muscle fibers and invade them early.

### *Identity of the Bacillus.*

From other anaerobes than *Bacillus welchii* the bacillus differs morphologically as *Bacillus welchii* does. It is needless to spend much time on this point. It also differs from others in the characters of its colonies. Further differences are given below.

*Bacillus fallax* is motile in culture under favorable conditions and highly motile in animal fluids. *Bacillus egens* is always absolutely immotile. *Bacillus fallax* has scarcely any pathogenic powers and these are quickly lost in subculture. *Bacillus egens* was markedly pathogenic after 6 weeks of subculture. *Fallax* is non-pathogenic for rabbits. When it does produce lesions in guinea pigs they are of a different character. The muscles are red and hyperemic and there

is no muscle lysis. Morphologically *fallax* is thinner, becomes Gram-negative more quickly, has more rounded ends, and does not produce long filaments as described for *Bacillus egens*.

*Bacillus welchii* differs fundamentally by its characteristic stormy fermentation of milk, its vigorous saccharolytic action on maltose, lactose, galactose, saccharose, and glycerol or inulin. Pathologically it does not produce such marked and extensive muscle lysis, and there is often hemorrhage in the lesions, giving a pink or red color to the affected muscles. It is not usually so pathogenic after long subculture, especially for rabbits. Morphologically it is characterized by the absence of filament formation under the conditions described for *Bacillus egens*. Its colonies are quite different.

*Bacillus œdemaciens* differs in morphology (chain formation in cultures and animal tissues; spore formation). In colony characteristics it is absolutely unlike *Bacillus egens*, and the pathological lesions are totally different. There is little or no muscle lysis, but an extensive white jelly-like edema.

*Vibrion septique* differs in motility, morphology, formation of long filaments in peritoneal fluid, spore formation, colony characters, and pathogenic action.

*Bacillus aerofœtidus* is practically non-pathogenic. It is a much smaller, thinner bacillus with much rounded ends, quickly becoming Gram-negative. Its colonies are different and it liquefies blood serum and ferments lactose vigorously.

*Bacillus egens* differs from all the other pathogenic anaerobes in the lack of spore formation and of proteolytic power.

The result of this case shows the importance of careful bacteriological control in testing the use of an anti-gas gangrene serum. It would have been easy to mistake the case for a *Bacillus welchii* infection, especially as *Bacillus welchii* was present on the wound surface, together with other aerobes and anaerobes. Of course the serum could not be expected to have therapeutic value in this particular case.

Whether such an infection as this will prove to be common is uncertain. Its presence would be extremely easy to overlook in the event of a coincident infection with *Bacillus welchii*, for *Bacillus egens* would add scarcely a single positive morphological or cultural character to mixed cultures.

*Nomenclature.*—Since the bacillus appears to be a new species, a name has been given it. Its most evident difference when compared to any other anaerobe consists in the lack of some characteristic; it differs from *Bacillus welchii* by lack of fermentative powers; from *fallax* by lack of motility; from *œdemaciens* and other anaerobes by lack of spore formation and of proteolytic power. Therefore the name *egens* has been chosen.

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# SPIROCHÆTA HEBDOMADIS, THE CAUSATIVE AGENT OF SEVEN DAY FEVER (NANUKAYAMI).

## Second Paper.

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### INTRODUCTION.

Etiological studies on *nanukayami*, or seven day fever, have been conducted by us in the Prefecture of Fukuoka since the year 1915. In November, 1916, we observed a certain spirochete in a guinea pig which had received an inoculation of blood from a patient having this disease. The spirochete resembled *Spirochæta icterohæmorrhagiæ* in form and motion, but the results of animal experiments and immunological studies undertaken at that time showed it to be a new species and the causative agent of seven day fever. Hence we named it *Spirochæta hebdomadis*.

We have come to know since that this disease occurs not only in the Prefecture of Fukuoka, but also in those of Shizuoka, Okayama, Shiga, Nara, Hyogo, Hiroshima, Kochi, in Kyoto, and elsewhere. These facts were reported in April, 1917, at the meetings of the Nippon Medical Society, the Nippon Pathological Society, and the Nippon Hygienic Society.[1] But the cases of seven day fever which had come under our observation up to that time were few in number, and we were obliged to wait until the next epidemic season of the disease, which was the autumn of that year, in order to continue our investigation.

On September 7, 1917, we went to Sawara County in the Prefecture of Fukuoka. Here by animal experiments made in a number of cases

[1] *Nippon Naika Gakkai Zasshi*, 1917, v, No. 5.

of seven day fever we succeeded in establishing the presence of *Spirochæta hebdomadis* in the blood and in making the discoveries here reported.

A summary of the report made in April, 1917, regarding the causative agent of seven day fever follows:

1. Since 1915, animal experiments had been carried on with six cases of seven day fever. In two cases we used patients' blood taken on the 3rd and 4th days of the disease, in three cases blood from the 6th day, and in one case from the 8th day. The animals employed were rabbits, guinea pigs, white rats, and mice. Three guinea pigs, which had received injections of blood from a patient on the 6th day of the disease, developed fever and died. Spirochetes were found in their livers.

2. The spirochete found resembled *Spirochæta icterohæmorrhagiæ* in form and motion, but the results of animal experiments and immunological studies proved it to be a new species.

3. When a pure culture of this spirochete was inoculated into guinea pigs, they showed symptoms resembling those of seven day fever.

4. In the serum of a patient recovering from seven day fever immune bodies were found which killed and dissolved the spirochete.

5. We proceeded to search for the animal host harboring the organism and discovered the spirochete in the kidneys of wild mice (*Microtus montebelli*). In other words, it was found that wild mice are the carriers of the spirochete, and that they are constantly disseminating it through their urine.

6. There is a close relation between the districts infected with seven day fever and the regions infested with wild mice.

Of all these points, we place the greatest importance upon the immunological observations. The spirochete under discussion, as has been stated, closely resembles *Spirochæta icterohæmorrhagiæ* in form and motility. By means of Pfeiffer's test, however, we found that it was not affected by horse serum immunized with *Spirochæta icterohæmorrhagiæ* or by the serum of a patient convalescing from spirochætosis icterohæmorrhagica. On the other hand, the serum of goats immunized with this spirochete or the serum of a patient recovering from seven day fever failed to act upon *Spirochæta icterohæmorrhagiæ*.

We injected the two immunized sera into guinea pigs infected with either seven day fever or spirochætosis icterohæmorrhagica. The animals inoculated with the homologous immune serum recovered and no spirochetes could be found in their blood, while those

injected with the heterologous immune serum met with the characteristic death of the disease. The spirochetes in the blood were in no way affected by the serum. We inoculated the corresponding spirochetes into guinea pigs which had recovered from seven day fever or spirochætosis icterohæmorrhagica and found that they were not affected, while the animals inoculated with heterologous spirochetes contracted the corresponding disease.

From these observations and on the basis of the modern immunological point of view, we concluded that the spirochete under discussion represents a species different from *Spirochæta icterohæmorrhagiæ*.

We succeeded, therefore, in confirming the causative agent of seven day fever. But in the six cases of the disease which we observed, we were able to confirm the presence of the spirochete in only one. This was probably attributable to the fact that *Spirochæta hebdomadis*, in contrast to *Spirochæta icterohæmorrhagiæ*, does not affect all guinea pigs to the same degree. In heavy animals we found that the inoculation of liver emulsion or pure culture containing even a large number of the spirochetes would not uniformly lead to infection. Only in light weight guinea pigs were we able to produce experimental seven day fever by inoculation. The conclusion was based on the results of the animal experiments referred to, but to test its validity it was necessary that it should be supported by further experiments, with guinea pigs of light weight. As the disease occurs only in the autumn, we had no opportunity to conduct experiments until our visit to Sawara County in 1917.

## EXPERIMENTAL.

The present researches in Sawara County covered the villages of Hara, Kanetake, Takuma, and Iki.[2] The cases of seven day fever which came under our observation from September 7, 1917, to the beginning of November, 1917, numbered twenty-six in all. We proceeded to the infected districts every other day and often made more than one animal experiment on the same patient. Serum was collected when necessary. Of the twenty-six patients under obser-

[2] The patients were studied through the kindness of Dr. Matsuguchi, Dr. Morita, and Dr. Tani of the district.

TABLE I.

*Guinea Pigs Inoculated with the Blood of Seven Day Fever Patients.*

| Case No. | Age. | Sex. | Day of first examination. | Length of time after onset. | No. of guinea pigs used. | Weight of animals. | Results. |
|---|---|---|---|---|---|---|---|
| | *yrs.* | | *1917* | *days* | | *gm.* | |
| 1 | 26 | Male. | Sept. 28 | 1 | 2 | 95 | + |
| | | | | | | 65 | + |
| 2 | 32 | " | Oct. 7 | 2 | 2 | 90 | + |
| | | | | | | 130 | + |
| 3 | 25 | " | Sept. 7 | 3 | 2 | 115 | + |
| | | | | | | 135 | + |
| 4 | 19 | " | " 13 | 3 | 2 | 116 | + |
| | | | | | | 118 | + |
| 5 | 34 | " | " 20 | 3 | 2 | 107 | + |
| | | | | | | 110 | + |
| 6 | 17 | " | " 26 | 3 | 3 | 100 | + |
| | | | | | | 105 | + |
| | | | | | | 105 | + |
| 7 | 20 | " | Oct. 5 | 3 | 2 | 95 | + |
| | | | | | | 110 | + |
| 8 | 31 | " | " 5 | 3 | 1 | 120 | + |
| 9 | 26 | " | " 5 | 3 | 2 | 100 | + |
| | | | | | | 110 | + |
| 10 | 18 | " | " 7 | 3 | 2 | 95 | + |
| | | | | | | 110 | + |
| 11 | 10 | " | Sept. 22 | 4 | 1 | 105 | + |
| 12 | 31 | " | " 26 | 4 | 2 | 100 | + |
| | | | | | | 110 | + |
| 13 | 42 | " | " 28 | 4 | 2 | 85 | + |
| | | | | | | 100 | + |
| 14 | 23 | " | Oct. 5 | 4 | 1 | 100 | + |
| 15 | 18 | Female. | " 9 | 4 | 1 | 140 | − |
| 16 | 16 | Male. | Nov. 5 | 4 | 3 | 90 | + |
| | | | | | | 115 | + |
| | | | | | | 155 | + |
| 17 | 16 | " | Sept. 7 | 5 | 2 | 98 | + |
| | | | | | | 109 | + |
| 18 | 43 | " | " 24 | 5 | 1 | 110 | + |
| 19 | 31 | " | Oct. 5 | 5 | 2 | 95 | + |
| | | | | | | 150 | + |
| 20 | 23 | " | Sept. 11 | 6 | 4 | 85 | + |
| | | | | | | 110 | + |
| | | | | | | 115 | + |
| | | | | | | 118 | − |
| 21 | 17 | " | " 16 | 7 | 4 | 133 | − |
| | | | | | | 142 | − |
| | | | | | | 149 | − |
| | | | | | | 114 | − |
| 22 | 16 | " | Oct. 5 | 7 | 2 | 97 | + |
| | | | | | | 105 | − |
| 23 | 20 | " | " 7 | 7 | 2 | 110 | − |
| | | | | | | 150 | − |

vation, we collected blood from twenty-three. The blood was injected into the peritoneal cavity of guinea pigs ranging in weight from 65 to 150 gm.; most of the animals averaged 100 gm. At least two animals were used for each test, for being small and light they frequently died in the course of the experiment. The quantity of blood injected was about 1 cc. for every 100 gm. of the animal's weight. Table I shows the results obtained from the twenty-three cases.

The twenty-three cases include one of blood taken on the 1st day of the disease, one on the 2nd day, eight on the 3rd day, six on the 4th day, three on the 5th day, one on the 6th day, and three on the 7th day. The guinea pigs inoculated with blood from Case 15, taken on the 4th day, and from Cases 21 and 23 taken on the 7th day, failed to develop fever and the experiments were negative. In the other twenty cases, however, the inoculated guinea pigs all developed fever in 5 to 7 days, and spirochetes were found in the blood. They were isolated in every case, almost in pure culture.

Daily records were kept of the temperatures of the guinea pigs. We also examined by dark-field illumination spirochetes in blood drawn from the ears, to ascertain whether the guinea pigs were affected. The animals were observed closely for symptoms. Only a few of the guinea pigs had icterus, and when it did occur, it was slight, far less severe than in spirochætosis icterohæmorrhagica. The animals were much less apt to bleed. In some of the guinea pigs the spirochetes disappeared from the blood in the course of time; the congestion also disappeared from the orbital conjunctivæ, the temperature fell, and the animals were restored to perfect health. Results such as these have not been observed in animal experiments with *Spirochæta icterohæmorrhagiæ*.

It has been stated already that heavy guinea pigs, those weighing more than 200 gm., are rarely infected with the spirochetes of seven day fever, and even when infected, they seldom die of the disease. This was the finding when one animal was inoculated from another. It remained to be proved, however, whether it was also true of guinea pigs inoculated directly with the blood of patients. We determined to test this point, using guinea pigs of different weights, as shown in Table II, and injecting them with quantities of blood in accordance

with the weight of the animal. The results show that with direct inoculation also the smaller and lighter animals were more easily affected than the heavier ones. Even heavy animals were occasionally affected by the disease, but the percentage was far smaller than with lighter ones. This finding proves that our hypothesis as to the influ-

TABLE II.

*Guinea Pigs of Different Weights Inoculated with the Blood of Seven Day Fever Patients.*

| Experiment No. | Guinea pig No. | Weight. | Amount of blood injected intraperitoneally. | Results. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Spirochetes in blood. | Course. | Autopsy. | Spirochetes in liver. | Spirochetes in kidney. |
| | | *gm.* | *cc.* | | | | | |
| 1 | 1 | 115 | 2.0 | + | Died on 10th day. | + | 5–6 in field. | |
| 2 | 2 | 118 | 2.0 | − | Normal. | | | |
| 3 | 3 | 110 | 2.0 | − | " | | | |
| 4 | 4 | 85 | 2.0 | + | Died on 10th day. | + | 10 in field. | |
| 5 | 5 | 90 | 1.0 | + | Died on 6th day. | + | 10 in field. | |
| 6 | 6 | 95 | 1.0 | − | " " 2nd " | − | — | |
| 7 | 7 | 115 | 1.2 | + | " " 7th " | + | 3–4 in field. | |
| 8 | 8 | 155 | 1.5 | + | " " 8th " | + | 1–2 " " | |
| 9 | 9 | 210 | 2.0 | − | " " 16th " | − | — | — |
| 10 | 10 | 290 | 3.0 | − | " " 15th " | + | -- | 1 in 1 specimen. |
| 11 | 11 | 305 | 3.0 | − | " " 4th " | − | — | |
| 12 | 12 | 135 | 1.0 | − | Died on 9th day. | + | 1 in 20–25 fields. | |
| 13 | 13 | 150 | 1.5 | − | " " 9th " | ± | — | |
| 14 | 14 | 185 | 2.0 | − | " " 6th " | − | — | |
| 15 | 15 | 475 | 5.0 | − | " " 10th " | + | 1 in 20–25 fields. | |
| 16 | 16 | 625 | 6.0 | + | " " 29th " | + | — | 1 in 1 specimen. |

ence of weight was in the main correct. When inoculation is carried on in a series from animal to animal, the smaller animals are preferable.

The animal experiments demonstrate that the spirochete under discussion is not the same as *Spirochæta icterohæmorrhagiæ*, but that it agrees in every respect with *Spirochæta hebdomadis*, the discovery of

which we have reported.[3] There is no room left for doubt that the organism obtained from these twenty cases was *Spirochæta hebdomadis*, but for further proof immunological experiments were undertaken. Serum was collected from the twenty patients in the stage of convalescence. This serum, together with the spirochete obtained in 1916 from a patient having seven day fever, and also *Spirochæta icterohæmorrhagiæ*, was subjected to Pfeiffer's test.

Pfeiffer's tests were made in each of the twenty cases. As the results were identical in all, we have reported only five (Tables III and IV). The tests show that the serum of these patients had no effect on *Spirochæta icterohæmorrhagiæ*, while it killed and dissolved *Spirochæta hebdomadis*. It is evident, therefore, that the serum contained immune bodies.

We carried out Pfeiffer's test with the serum from convalescent patients having seven day fever, which contained strong immune bodies both against the spirochetes obtained in 1917 and those of the year previous, and immune serum of *Spirochæta icterohæmorrhagiæ*. As this experiment requires a comparatively large number of guinea pigs, it was confined to ten cases selected from the total number of twenty. We found that the spirochetes of the ten cases were not affected by the immune serum of *Spirochæta icterohæmorrhagiæ*, while the organisms were killed and dissolved by serum obtained from convalescent cases of seven day fever. This serum had already been shown to contain strong immune bodies against the spirochete of 1916.

Pfeiffer's tests were made in ten cases. The results being identical in all, we shall give the records for two only (Tables V and VI).

We believe that the animal experiments and the immunological studies made have proved conclusively that the organisms found in the twenty cases observed in 1917 differ from *Spirochæta icterohæmorrhagiæ*, and that they are of precisely the same species as *Spirochæta hebdomadis* discovered by us the previous year. Hence we are justified in concluding that the causative agent of seven day fever is undoubtedly *Spirochæta hebdomadis* and that the presence of this organism can always be proved by inoculating blood taken

[3] Ido, Y., Ito, H., and Wani, H., *J. Exp. Med.*, 1918, xxviii, 435.

TABLE III.

*Pfeiffer's Tests. Spirochæta hebdomadis Obtained in 1916 and Convalescent Serum from Seven Day Fever.*

| Guinea pig No. | Weight. | Intraperitoneal injection. | | Spirochetes in peritoneal fluid. | | Course. | Results. |
|---|---|---|---|---|---|---|---|
| | | Serum used. | Spirochetes. | After 30 min. | After 2 hrs. | | |
| | *gm.* | | | | | | |
| Control. | 85 | Isotonic salt solution, 1 cc. | *S. hebdomadis*, 1 cc. | 1–2 in field. | 1 in field. | No protection. | – |
| " | 87 | Immune horse, icterohemorrhagic, 1 cc. | *S.* " 1 " | 1–2 " " | 1–2 " " | " " | – |
| " | 143 | Immune, patient's, icterohemorrhagic, 1 cc. | *S.* " 1 " | 1 " " | 1 " " | " " | – |
| " | 104 | Immune, seven day fever (Case 24), 1 cc. | *S.* " 1 " | 0 " specimen. | 0 " specimen. | Protection. | + |
| 17 | 103 | Immune, seven day fever (Case 17), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |
| 18 | 115 | Immune, seven day fever (Case 3), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |
| 19 | 117 | Immune, seven day fever (Case 20), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |
| 20 | 107 | Immune, seven day fever (Case 12), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |
| 21 | 102 | Immune, seven day fever (Case 4), 1 cc. | *S.* " 1 " | 0 " " | 0 " " | " | + |

TABLE IV.

*Pfeiffer's Tests. Spirochæta icterohæmorrhagiæ and Convalescent Serum from Seven Day Fever.*

| Guinea pig No. | Weight. | Intraperitoneal injection. | | Spirochetes in peritoneal fluid. | | Course. | Results. |
|---|---|---|---|---|---|---|---|
| | | Serum used. | Spirochetes. | After 30 min. | After 2 hrs. | | |
| | *gm.* | | | | | | |
| Control. | 150 | Isotonic salt solution, 1 cc. | *S. icterohæmorrhagiæ*, 10 in field, 1 cc. | 4–5 in field. | 4–5 in field. | No protection. | – |
| " | 135 | Immune, patient's, ictero-hemorrhagic, 1 cc. | " " | 0 " specimen. | 0 " specimen. | Protection. | + |
| 22 | 120 | Immune, seven day fever (Case 17), 1 cc. | " " | 4–5 " field. | 2–3 " field. | No protection. | – |
| 23 | 160 | Immune, seven day fever (Case 3), 1 cc. | " " | 3–4 " " | 2–3 " " | " " | – |
| 24 | 170 | Immune, seven day fever (Case 20), 1 cc. | " " | 3–4 " " | 1–2 " " | " " | – |
| 25 | 180 | Immune, seven day fever (Case 12), 1 cc. | " " | 1–2 " " | 2–3 " " | " " | – |
| 26 | 155 | Immune, seven day fever (Case 4), 1 cc. | " " | 1–2 " " | 1–2 " " | " " | – |

TABLE V.

*Pfeiffer's Tests. Spirochetes from Case 4, and Immune Serum of Spirochæta hebdomadis and Spirochæta icterohæmorrhagiæ.*

| Guinea pig No. | Weight. | Intraperitoneal injection. | | Spirochetes in peritoneal fluid. | | Course. | Results. |
|---|---|---|---|---|---|---|---|
| | | Serum used. | Spirochetes. | After 30 min. | After 2 hrs. | | |
| | *gm.* | | | | | | |
| Control. | 95 | Isotonic salt solution, 1 cc. | Spirochetes (Case 4), 10 in field, 1 cc. | 1–2 in field. | 1 in 1–2 fields. | No protection. | − |
| " | 107 | Case 4, 1 cc. | " " | 0 in specimen. | 0 " specimen. | Protection. | + |
| 27 | 110 | Immune, seven day fever (Case 25), 1 cc. | " " | 0 " " | 0 " " | " | + |
| 28 | 110 | Immune, seven day fever (Case 26), 1 cc. | " " | 0 " " | 0 " " | " | + |
| 29 | 88 | Immune, horse, ictero-hemorrhagic, 1 cc. | " " | 1 " 1–2 fields. | 1 " 1–2 fields. | No protection. | − |
| 30 | 100 | Immune, patient's, ictero-hemorrhagic, 1 cc. | " " | 1 " 1–2 " | 1 " 1–2 " | " " | − |

TABLE VI.

*Pfeiffer's Tests. Spirochetes from Case 17, and Immune Serum of Spirochæta hebdomadis and Spirochæta icterohæmorrhagiæ.*

| Guinea pig No. | Weight. | Intraperitoneal injection. | | Spirochetes in peritoneal fluid. | | Course. | Results. |
|---|---|---|---|---|---|---|---|
| | | Serum used. | Spirochetes. | After 30 min. | After 2 hrs. | | |
| | *gm.* | | | | | | |
| Control. | 94 | Isotonic salt solution, 1 cc. | Spirochetes (Case 17), 10 in field, 1 cc. | 3–4 in 1 line.* | 1 in a few fields. | No protection. | − |
| " | 105 | Case 17, 1 cc. | " " | 0 " specimen. | 0 " specimen. | Protection. | + |
| 31 | 103 | Immune, seven day fever (Case 25), 1 cc. | " " | 0 " " | 0 " " | " | + |
| 32 | 120 | Immune, horse, ictero-hemorrhagic, 1 cc. | " " | 1 " 7–8 fields. | 2–3 " 1 line. | No protection. | − |

* Line means from one side to the other of the cover-glass (65 to 70 fields).

from a patient at a proper stage of the disease into guinea pigs of light weight.

In cooperation with Doctors Okuda and Kishimoto we conducted experiments to ascertain the particular day after the onset of the disease when the blood of patients is infective for guinea pigs. Blood was taken from all the patients, usually every other day, for a period of 10 days from the onset. The weight of the animals used for experiment and the method of inoculation were the same as already described. The results are given in Table VII.

TABLE VII.

*Guinea Pigs Inoculated with Blood from 23 Cases of Seven Day Fever.*

| Length of time after onset. | No. of guinea pigs. | Positive. | Negative. | Doubtful. | Per cent positive. | Guinea pigs developing spirochætosis icterohæmorrhagica. | |
|---|---|---|---|---|---|---|---|
| | | | | | | No. of guinea pigs. | Per cent positive. |
| *days* | | | | | | | |
| 1 | 1 | 1 | 0 | 0 | 100 | | |
| 2 | 1 | 1 | 0 | 0 | 100 | 40 | 100 |
| 3 | 8 | 8 | 0 | 0 | 100 | | |
| 4 | 6 | 5 | 1 | 0 | 83.3 | | |
| 5 | 4 | 4 | 0 | 0 | 100 | 7 | 88.9 |
| 6 | 7 | 2 | 4 | 1 | 33.3 | 19 | 73.7 |
| 7 | 12 | 1 | 8 | 3 | 11.1 | 15 | 46.7 |
| 8 | 5 | 0 | 4 | 1 | 0 | 7 | 13.3 |
| 9 | 1 | 0 | 1 | 0 | 0 | 6 | 16.7 |
| 10 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| Totals ... | 46 | | | | | 95 | |

Up to the 3rd day from the onset of the disease ten cases were positive; *i.e.*, 100 per cent of the animals inoculated contracted the disease. On the 4th day some negative results appeared. On the 5th day all four cases were positive; the inoculation experiment showed 100 per cent positive. On the 6th day the percentage of guinea pigs infected with the disease fell considerably, being only 33.3. On the 7th day only 11.1 per cent were positive. On and after the 8th day all were negative, as may be seen in Table VII. The fact that after the 4th day of the disease the experiments on blood inoculation showed negative results may be explained as being attrib-

utable to the appearance in the blood of immune bodies against *Spirochæta hebdomadis*.

An experiment was made to ascertain the time of appearance in the blood of patients of immune bodies which kill *Spirochæta hebdomadis*. Table VIII shows the results.

It will be seen from the table that up to the 5th day Pfeiffer's test failed to prove the appearance of immune bodies. On the 6th and 7th days, however, the test showed the appearance of a large quantity of immune bodies, and on and after the 8th day immunity was complete. Pfeiffer's tests were made, as with *Spirochæta icterohæmorrhagiæ*, with serum obtained from patients and *Spirochæta hebdomadis*. The imperfect immunity referred to in Table VIII

TABLE VIII.

*Time of Appearance of Immune Bodies in the Blood of Seven Day Fever Patients.*

| Length of time after onset. | Positive reactions. | Incomplete reactions. | Negative reactions. | Totals. |
|---|---|---|---|---|
| *days* | | | | |
| 3 | | | 1 | 1 |
| 4 | | | 2 | 2 |
| 5 | | | 2 | 2 |
| 6 | 1 | 4 | 1 | 6 |
| 7 | 7 | 1 | 2 | 10 |
| 8 | 9 | | | 9 |
| 9 | 5 | | | 5 |
| 10 and over. | 2 | | | 2 |

concerns two cases of seven day fever. For Pfeiffer's test in these cases the intraperitoneal fluid collected after 30 minutes and 2 hours respectively was examined by dark-field illumination. In one it was found to differ wholly from control experiments, for after 2 hours no spirochetes could be seen, although the guinea pig was later experimentally infected with the disease. In the other the intraperitoneal fluid also differed from the control. Spirochetes were not completely dissolved and even after 2 hours some of the organisms were still moving slightly.

It is evident that the blood in these cases had begun to lose its infecting power on the 6th day from the onset of the disease. Hence it was to be expected that on and after the 8th day it had become completely negative.

*Spirochæta hebdomadis*, like *Spirochæta icterohæmorrhagiæ*, is found occasionally, although in small numbers only, in film preparations of the blood of patients. We have observed it in four preparations stained by Giemsa's method, one of which was prepared on the 2nd day, two on the 3rd, and one on the 4th day of the disease.

With the cooperation of Doctors Okuda and Kishimoto we ascertained further that the spirochetes of seven day fever are excreted by patients in the urine. A quantity of 40 cc. of urine, taken aseptically from a patient, was centrifugalized and the deposit examined by dark-field illumination. When no spirochetes were observed the deposit was injected into the peritoneal cavity of guinea pigs. Usually after the 8th day of the disease microscopic examination showed the presence of spirochetes in the urine of patients having seven day fever. The only exception was a case where they were found on the 4th day. In the other twenty-one cases examined they were not observed until the 8th day. After the 9th day they were found in comparatively large numbers, and from about the 18th or 19th until the 25th day we found them uniformly. After that day the percentage decreased gradually, but as late as the 39th day we observed active spirochetes. We found a similar condition in spirochætosis icterohæmorrhagica, although in seven day fever the period in which the spirochetes are discharged is somewhat longer. In 40 cases microscopic examination failed to disclose spirochetes. In all these an injection of the deposit was made into the peritoneal cavity, but only two guinea pigs became infected.

Seven day fever is a disease found not only in the Prefecture of Fukuoka, but in other districts of Japan as well. We have established the fact that the "autumnal epidemic" in Shizuoka Prefecture, "*sakushu* fever" in Okayama Prefecture, "autumnal fever" in Kochi Prefecture, a fever found in Kyoto and in the Prefectures of Shiga, Hyogo, Nara, Hiroshima, Miyazaki, Nagasaki, and Formosa are in reality the same disease; *i.e.*, seven day fever. This statement is based on immunological investigations of the serum of patients.[4]

[4] Reported in April, 1917, at the meeting of the Nippon Naika Gakkai.

## CONCLUSIONS.

1. *Spirochæta hebdomadis* is always present in seven day fever and can be confirmed by animal experiments with guinea pigs of light weight.

2. The causative agent of this disease can also be found in film preparations of the blood of patients, though it is not present in large numbers.

3. *Spirochæta hebdomadis* is discharged in the urine of patients having seven day fever. The number of spirochetes in the urine is great during convalescence.

4. Seven day fever is a disease found not only in the Prefecture of Fukuoka, but in many other districts of Japan as well.

We take pleasure in expressing our indebtedness to Professor Inada, under whose direction the studies were undertaken. We also desire to thank our colleagues, Doctors Okuda and Kishimoto, for their assistance.

The studies here reported were carried on in the Laboratory for the Serum Therapy of Spirochætosis Icterohæmorrhagica in connection with Dr. Inada's clinic, established by the Association Commemorating the Tercentenary Anniversary of Toshogu. The experimental animals used were supplied by the Association. We extend our thanks to Prince Iesato Tokugawa, the President of the Association.

# THE USE OF THE FINAL HYDROGEN ION CONCENTRATION IN DIFFERENTIATION OF STREPTOCOCCUS HÆMOLYTICUS OF HUMAN AND BOVINE TYPES.

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*Streptococcus hæmolyticus* is known to cause certain pathologic conditions in man, such as erysipelas, puerperal sepsis, cellulitis, septic sore throat, bronchopneumonia, etc. It is frequently associated with other diseases, often as a secondary invader in measles and scarlet fever. A hemolytic streptococcus of similar characteristics is also found as the etiologic agent in many diseases of lower animals, particularly in mastitis of cows. Furthermore, an organism of like character is almost constantly present in milk and dairy products. In milk-borne epidemics of septic sore throat the weight of evidence supports the view that the disease is caused by human types of hemolytic streptococci which are accidently communicated to the udder of the cow, rather than to the streptococci which are the common cause of bovine mastitis, and which are so frequently found in milk when no disease is present. From an epidemiological standpoint the recognition of distinctive human and bovine types of this organism is of considerable interest and practical importance.

The occurrence of a hemolytic streptococcus, resembling to such a degree the organism found in pathologic lesions of man, has made necessary the devising of methods for differentiation of this type of streptococcus from that of human origin.

The bacteriological diagnosis of these types rests upon the summation of a number of differential features, rather than upon the dis-

tinctive value of any one test. Minor morphological and cultural characters, differences in the thermal death-point, and variations in the degree of hemolytic activity and pathogenicity for rabbits serve to indicate that essential biologic differences exist between these two types. In addition, many observers have noted the fact that non-pathogenic hemolytic streptococci produce more acid in carbohydrate media than the pathogenic varieties. These observations have been based upon the titratable acidity recorded in terms of the amount of normal alkali required to neutralize a given amount of culture fluid.

Some work has been done on the final hydrogen ion concentration as a means of differentiation of these organisms. Clark (1), discussing the diagnostic value of the final hydrogen ion concentration in differentiating cultures of *Bacillus coli*, states: "Sufficient work has been done in this laboratory to indicate that the same phenomena . . . . apply to many of the streptococci." Ayers (2), studying 200 cultures of streptococci, found that two final zones of hydrogen ion concentration were reached: pH. 4.6 to 4.8, 5.5 to 6.0. Smillie (3) records five strains tested by this method, but does not lay stress upon the results obtained. Ayers, Johnson, and Davis (4) have published interesting observations on the limiting hydrogen ion concentration of a series of streptococci, and have found that in dextrose-yeast-peptone medium the strains from pathogenic sources have a lower limiting hydrogen ion concentration than that reached by the non-pathogenic forms. They emphasize again the fact that titratable acidity, as a measure of the fermentative activity of an organism, is influenced so much by the nature and buffer content of the culture medium that this method should be supplanted by the determination of the hydrogen ion concentration. Ayers[1] has suggested the use of the limiting hydrogen ion concentration in differentiation of human and bovine types of streptococci and has found that the organisms obtained from milk gave the high limiting hydrogen ion concentration, that is from pH 4.5 to 5.0, and that most of the cultures were hemolytic.

The present work is confined to a study of hemolytic streptococci, and from it there has developed the fact that the hydrogen ion con-

[1] Personal communication.

centration at which the human strains of *Streptococcus hæmolyticus* cease to grow is different from that which limits growth of hemolytic cultures from bovine sources.

## EXPERIMENTAL.

*Source of Cultures.*—This study comprises 169 strains of *Streptococcus hæmolyticus*, of which 124 were of human origin and 45 from bovine sources. Among the latter are included a number of strains recovered directly from mastitis in cows and others isolated from milk. Two strains are included among these from bovine sources, which were isolated by Smith and Brown (5) from udders of cows during Boston epidemics of septic sore throat, and identified by them as of human type.[2] Nineteen strains came from cream cheese of different brands.

The strains of hemolytic streptococci of human origin were in the majority of instances isolated from the throats, sputum, blood, and lung lesions of men suffering from postmeasles bronchopneumonia, or from the throats of those intimately in contact with these patients during an epidemic of pneumonia at one of the camp hospitals. In addition, several strains of *Streptococcus hæmolyticus* from erysipelas, postpartum sepsis, meningitis, and cellulitis have been included.[3]

*Methods of Isolation and Identification.*—All the cultures were isolated and identified according to the standard methods for the isolation and identification of *Streptococcus hæmolyticus* adopted by the Medical Department of the United States Army. Isolation was made from a single colony on plain blood agar, and subsequent identification confirmed by the hemolysin test, the bile reaction, and the fermentation of certain test substances, as inulin, salicin, etc. The original stock cultures were preserved in plain blood agar stabs or plain blood broth.

[2] The authors acknowledge their indebtedness to Dr. J. Howard Brown and Dr. Frederick S. Jones, of the Department of Animal Pathology of The Rockefeller Institute, Princeton, N. J., for many of these cultures.

[3] Dr. Charles Krumwiede, of the Department of Health of the City of New York, has furnished many of these cultures, and the authors wish to express their appreciation of this courtesy.

*Medium.*—The medium used for the determination of the final hydrogen ion concentration was meat infusion broth with the addition of 1 per cent dextrose. This medium was prepared from the infusion of fresh beef and contained 1 per cent peptone, 0.5 per cent salt, and 1 per cent dextrose. The initial reaction varied from pH 7.6 to 7.8. With the exception of the dextrose, the medium was the same as that used in this laboratory for the routine cultivation of pneumococcus, and the method of preparation was identical with that described by Avery, Chickering, Cole, and Dochez (6).

In all the experiments measured quantities of the dextrose broth were inoculated with uniform amounts of an 18 hour plain broth culture of the organism to be tested and incubated at 37°C.

*Determination of Hydrogen Ion Concentration.*

The colorimetric or indicator method of determining the hydrogen ion concentration of bacterial cultures is, because of its simplicity and availability, the method of choice in studies of the fermentative activity of organisms. The principles and application of this method have been so thoroughly set forth by Michaelis (7) and by Clark and Lubs (8), as well as by other authors, that reference will be made only to the most important details. The color of the culture medium and the turbidity produced by the massive growth of organisms, as well as by precipitation of proteins, have been the principal sources of error. This changing or masking of the indicator color may be obviated in two ways. First, it must be remembered that all successful media are buffer solutions; that is, they contain salts that require relatively large changes in acid or base to produce small changes in the reaction. The hydrogen ion concentration of these solutions is not materially changed by the addition of water. It is possible, therefore, to dilute the color of the medium itself without affecting the reaction. Secondly, the color of the medium may be superimposed upon that of the indicator by the comparator method introduced by Walpole (9) and adopted by Clark and Lubs. The intense turbidity of cultures of *Streptococcus hæmolyticus* grown in dextrose broth may be reduced by use of the supernatant culture fluid, although the combination of the dilution and comparator methods is usually sufficient to insure an accuracy of pH ± 0.1.

The hydrogen ion concentration determinations were made as follows: Duplicate tubes containing 5 cc. each of the culture were diluted with redistilled water to 15 cc. One drop of a 0.1 per cent alcoholic solution of methyl red was then added to one tube while the other tube was used for the comparator color. The hydrogen ion concentration was then read in the comparator block, as modified by Dernby and Avery (10), and recorded to the nearest tenth of a pH. With this indicator the cultures with a reaction more acid than a pH of 4.5 showed a definite claret-red color, while those less acid than a pH of 5.0 showed only a slight salmon tint. This difference is striking. It is important to use the indicator in sufficiently low concentration. The final reaction of the more acid group of organisms is about the lower limit of usefulness of methyl red, that is pH of 4.3, and too much indicator makes it impossible to distinguish between 4.6 and 4.3. Our experience shows that one small drop, about 0.02 cc., gave the sharpest reading. If, however, four or five times as much methyl red is used, the difference in color tints within this narrow zone is much less distinctive. This point cannot be overemphasized, especially since there is a tendency for most workers to use an excess of indicator. The accuracy of the determinations in the range pH 4.6 to 4.0 was controlled in several experiments by the use of the indicator brom phenol blue (tetrabromophenolsulfonephthalein). The standard solutions of known hydrogen ion concentration were prepared for the range 5.8 to 8.0 from phosphates by Sörensen's technique, and for the range 4.0 to 5.8 from acetate by Walpole's directions. The phosphates had been previously standardized by the hydrogen electrode.

*Influence of Dextrose.*

Since Clark and Lubs (11), in using this method for differentiating the types of *Bacillus coli*, have shown that the amount of dextrose available for fermentation by the organism influenced the final reaction, and that if insufficient dextrose was used a reversion of reaction followed, it was necessary to determine the effect of varying concentrations of dextrose upon the final hydrogen ion concentration of *Streptococcus hæmolyticus*. Further, it was desired to determine whether or not the sugar present in the medium has any effect upon the reaction. The following experiment was therefore devised.

To plain broth of known hydrogen ion concentration, dextrose was added in quantities sufficient to make 0.5, 1, and 1.5 per cent dextrose concentration. The medium was incubated over night at 37°C. to test sterility and then inoculated with 0.1 cc. each of an 18 hour plain broth culture of *Streptococcus hæmolyticus*. Broth which had been fermented to remove muscle sugar was used in the same manner.[4] Samples containing 0.5, 1, and 1.5 per cent dextrose were

TABLE I.

*Influence of the Concetration of Dextrose on the Final Hydrogen Ion Concentration.*

100 cc. bottles inoculated with 0.1 cc. each of 18 hour plain broth cultures of Human 24, Bovine C57, and Cheese 4.

| Series No. | Culture medium. | Initial hydrogen ion concentration. | Hydrogen ion concentration. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 24 hrs. | | | 14 days. | | |
| | | | Human. | Bovine. | Cheese. | Human. | Bovine. | Cheese. |
| | | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| 1 | Plain broth. | 7.6 | 7.35 | 7.25 | 7.15 | 7.3 | 7.1 | 7.3 |
| 2 | " " + 0.5% dextrose. | 7.5 | 5.0 | 4.5 | 4.5 | 5.1 | 4.5 | 4.5 |
| 3 | " " + 1% " | 7.4 | 5.1 | 4.5 | 4.3 | 5.1 | 4.5 | 4.3 |
| 4 | " " + 1.5% " | 7.5 | 5.0 | 4.5 | 4.3 | 5.1 | 4.5 | 4.3 |
| 5 | Sugar-free broth + 0.5% dextrose. | 7.5 | 5.1 | 4.5 | 4.3 | | | |
| 6 | " " + 1% " | 7.5 | 5.1 | 4.5 | 4.3 | 5.1 | 4.5 | 4.3 |
| 7 | " " + 1.5% " | 7.5 | 5.0 | 4.5 | 4.2 | 5.1 | 4.5 | 4.2 |

prepared and inoculated in the same way. The hydrogen ion concentration of the media, after the preliminary sterilization and incubation and before inoculation, was controlled, and 5 cc. samples of the supernatant liquid were removed from the bottles 24 hours and 2 weeks after inoculation (Table I). It is evident from these results that the concentration of dextrose from 0.5 to 1.5 per cent gives the same hydrogen ion concentration, and further, that either plain broth or sugar-free broth is equally available for this purpose. We have, therefore, used throughout the series an ordinary 1 per cent dextrose broth.

[4] In the preparation of sugar-free broth the meat infusion, before the addition of peptone, is fermented by *B. coli* for 18 to 24 hours.

*Rate of Acid Production of Streptococcus hæmolyticus in 1 Per Cent Dextrose Broth.*

It was important to determine the rate of acid production and length of time required to reach the final limiting hydrogen ion concentration. Hence the following experiments were carried out.

1 per cent dextrose broth was prepared from sugar-free medium, as in the previous experiment, and 100 cc. samples were inoculated with two human strains, two cheese strains, and one bovine strain. The cultures were incubated at 37°C., and at definite intervals 5 cc. duplicate samples were removed for examination. The results are given in Table II and plotted in Text-fig. 1.

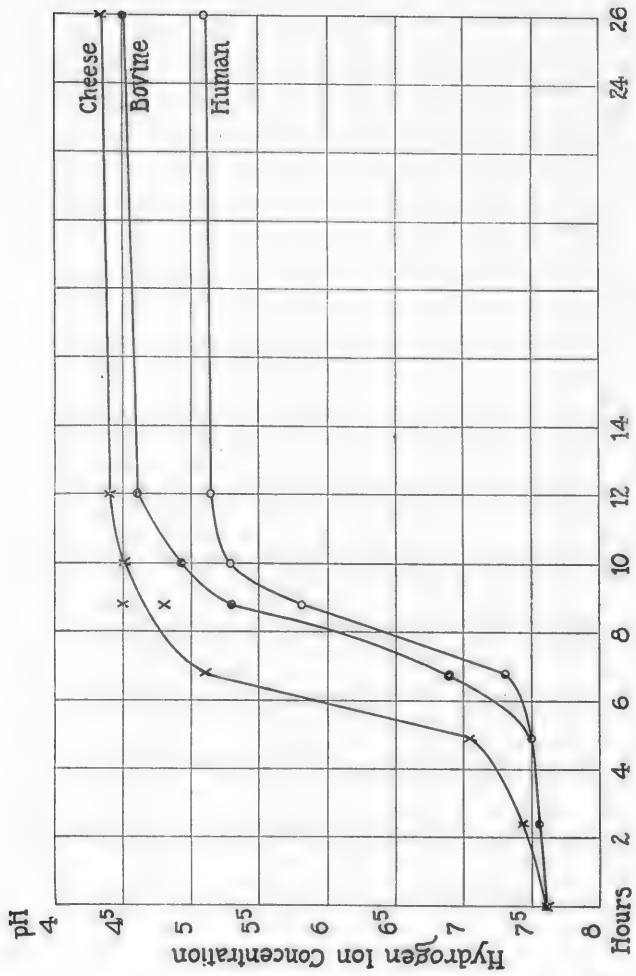
TABLE II.

*Rate of Acid Production.*

Sugar-free broth + 1 per cent dextrose. Initial pH = 7.6.

| Strain No. | Hydrogen ion concentration. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2½ hrs. | 4½ hrs. | 6½ hrs. | 9 hrs. | 10 hrs. | 12 hrs. | 26 hrs. | 15 days. |
| | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| Human 24 | 7.55 | 7.5 | 7.3 | 5.9 | 5.3 | 5.15 | 5.1 | |
| " 43 | 7.5 | 7.5 | 7.3 | 5.7 | 5.25 | 5.15 | 5.1 | |
| Cheese 64 | 7.45 | 7.05 | 5.1 | 4.8 | 4.5 | 4.3 | 4.3 | 4.3 |
| " 2 | 7.45 | 7.1 | 5.1 | 4.5 | 4.5 | 4.4 | 4.4 | |
| Bovine C57 | 7.5 | 7.5 | 6.9 | 5.3 | 4.95 | 4.6 | 4.5 | 4.4 |

It is evident from these results that the organisms from all three sources reached their final hydrogen ion concentration within 24 hours. The values for the duplicate cultures were so close that only one curve for each set is apparent. Although the curve for acid production of these organisms had reached the maximum within 24 hours, it seemed desirable to determine whether or not continued incubation produced any change in the hydrogen ion concentration. For this purpose nine strains were inoculated in 100 cc. portions of 1 per cent dextrose broth and incubated at 37°C. (Table III). These results substantiate the preceding experiments in that the final hydrogen ion concentration is reached within 24 hours, and show further that this final hydrogen ion concentration does not change on continued incubation. Although 14 days was the longest period




TEXT-FIG. 1. Rate of acid production of human and bovine types of *Streptococcus hæmolyticus*.

of test in this series, the same results have been obtained from cultures tested after 3 weeks.

TABLE III.

*Effect of Prolonged Incubation.*

100 cc. of sugar-free broth + 1 per cent dextrose inoculated with 0.05 cc. of 18 hour plain broth cultures. Initial pH = 7.3.

| Strain No. | Source. | Hydrogen ion concentration. | | | | |
|---|---|---|---|---|---|---|
| | | 18 hrs. | 2 days. | 4 days. | 7 days. | 14 days. |
| | | *pH* | *pH* | *pH* | *pH* | *pH* |
| 24 | Human. | 5.2 | 5.1 | 5.1 | 5.1 | 5.1 |
| 66 | " | 5.2 | 5.2 | 5.2 | 5.1 | |
| 2 | " | 5.1 | 5.1 | 5.1 | 5.1 | |
| 43 | " | 5.3 | 5.1 | 5.1 | 5.1 | |
| 14 | " | 5.2 | 5.1 | 5.15 | 5.1 | |
| 118 | " | 5.2 | 5.2 | 5.1 | 5.1 | |
| 276 | " | 5.2 | 5.1 | 5.1 | 5.1 | |
| 277 | " | 5.2 | 5.1 | 5.1 | 5.1 | 5.1 |
| 11 | " | 5.15 | 5.1 | 5.1 | 5.1 | 5.1 |

*Effect of Animal Passage upon the Final Hydrogen Ion Concentration.*

Since the question of virulence is always of first importance in a discussion of the biology of an organism, it was deemed advisable to determine whether or not animal passages had any effect upon the final hydrogen ion concentration. To determine this five original cultures and their corresponding subcultures, which had been passed through a number of animals, were selected and inoculated in 100 cc. portions of 1 per cent dextrose broth. The results are given in Table IV. It is evident from this experiment that the final hydrogen ion concentration of pathogenic *Streptococcus hæmolyticus* is not affected by animal passage.

*Constancy of the Final Hydrogen Ion Concentration of the Same Strains in Different Experiments.*

The constancy of the final hydrogen ion concentration of *Streptococcus hæmolyticus* is strikingly shown in Table V in which the hydrogen ion concentration of the same strains, but of different experiments, have been assembled.

TABLE IV.

*Influence of Animal Passage on the Final Hydrogen Ion Concentration.*

| Strain No. | No. of animal passages. | Hydrogen ion concentration. | | |
|---|---|---|---|---|
| | | 24 hrs. | 48 hrs. | 8 days. |
| | | *pH* | *pH* | *pH* |
| 1 | | 5.1 | 5.1 | 5.1 |
| 1 | 7 | 5.3 | 5.3 | 5.3 |
| 23 | | 5.1 | 5.1 | 5.1 |
| 23 | 16 | 5.15 | 5.1 | |
| 276 | | 5.15 | 5.1 | 5.1 |
| 276 | 25 | 5.1 | 5.1 | 5.1 |
| 24 | | 5.3 | 5.15 | 5.1 |
| 24 | 25 | 5.1 | 5.1 | 5.1 |
| 84 | | 5.1 | 5.1 | 5.1 |
| 84 | 16 | 5.3 | 5.3 | 5.3 |

TABLE V.

*Constancy of the Final Hydrogen Ion Concentration of the Same Strains in Different Experiments.*

1 per cent dextrose in all media.

| Experiment No. | Strain No. | | | | | |
|---|---|---|---|---|---|---|
| | Human. | | Bovine. | | Cheese. | |
| | 24 | 8 | C53 | C57 | 4 | 1 |
| | *pH* | *pH* | *pH* | *pH* | *pH* | *pH* |
| 1 | 5.1 | | | | | |
| 2 | | 5.2 | | | | |
| 3 | | | 4.4 | 4.4 | 4.3 | 4.3 |
| 4, *a* | 5.15 | 5.1 | | | | |
| 4, *b* | 5.1 | 5.3 | | | | |
| 5 | | | 4.3 | 4.3 | 4.3 | 4.3 |
| 6 | 5.1 | | | 4.5 | 4.3 | 4.3 |
| 7 | | | 4.5 | 4.5 | | |
| 8 | 5.1 | | | 4.5 | 4.3 | |
| 9 | 5.1 | | | 4.5 | 4.3 | |
| 10 | | | | | 4.5 | |

TABLE VI.

*Final Hydrogen Ion Concentration of Streptococcus hæmolyticus of Known Human Origin.*

| Strain No. | Source. | Diagnosis. | pH |
|---|---|---|---|
| 1 | Autopsy (lung). | Bronchopneumonia following measles. | 5.2 |
| 5 | " " | " " " | 5.1 |
| 20 | " " | " " " | 5.1 |
| 29 | Sputum. | " " " | 5.15 |
| 32 | Autopsy (lung). | " " " | 5.0 |
| 39 | " " | " " " | 5.2 |
| 59 | " (blood). | " " " | 5.1 |
| 78 | | " " " | 5.2 |
| 92 | Pleural fluid. | " " " | 5.2 |
| 93 | | " " " | 5.1 |
| 97 | Pleural fluid. | " " " | 5.1 |
| 107 | Sputum. | " " " | 5.3 |
| 110 | | " " " | 5.1 |
| 113 | | " " " | 5.1 |
| 118 | Pleural fluid. | " " " | 5.2 |
| 136 | | " " " | 5.0 |
| 137 | Throat. | " " " | 5.1 |
| 138 | Sputum. | " " " | 5.1 |
| 142 | | " " " | 5.1 |
| 145 | Throat. | " " " | 5.1 |
| 149 | Blood. | " " " | 5.1 |
| 151 | Pleural fluid. | " " " | 5.1 |
| 49 | Sputum. | " " German measles. | 5.1 |
| 2 | Autopsy (lung). | " | 5.1 |
| 3 | " " | " | 5.1 |
| 14 | " " | " | 5.1 |
| 24 | " " | " | 5.1 |
| 50 | Pleural fluid. | " | 5.1 |
| 55 | " " | " | 5.0 |
| 67 | Blood. | " | 5.1 |
| 72 | Throat. | " | 5.1 |
| 84 | Pleural fluid. | " | 5.2 |
| 139 | Throat. | " | 5.1 |
| 143 | Pleural fluid. | " | 5.2 |
| 152 | " " | " | 5.1 |

TABLE VI—*Continued.*

| Strain No. | Source. | Diagnosis. | pH |
|---|---|---|---|
| 10 | Throat. | Measles. | 5.1 |
| 37 | " | " | 5.0 |
| 43 | " | " | 5.1 |
| 47 | " | " | 5.1 |
| 53 | " | " | 5.1 |
| 60 | " | " | 5.1 |
| 64 | " | " | 5.1 |
| 66 | " | " | 5.2 |
| 71 | " | " | 5.1 |
| 79 | " | " | 5.1 |
| 82 | " | " | 5.0 |
| 83 | " | " | 5.1 |
| 85 | " | " | 5.1 |
| 86 | " | " | 5.1 |
| 88 | " | " | 5.1 |
| 89 | " | " | 5.1 |
| 96 | " | " | 5.2 |
| 98 | " | " | 5.2 |
| 108 | " | " | 5.1 |
| 116 | " | " | 5.2 |
| 117 | " | " | 5.1 |
| 119 | " | " | 5.0 |
| 126 | " | " | 5.2 |
| 127 | " | " | 5.1 |
| 128 | " | " | 5.1 |
| 141 | " | " | 5.1 |
| 148 | " | " | 5.15 |
| 150 | " | " | 5.1 |
| 4 | " | German measles. | 5.0 |
| 28 | " | " " | 5.1 |
| 38 | " | " " | 5.0 |
| 45 | " | " " | 5.1 |
| 47 | " | " " | 5.1 |
| 48 | " | " " | 5.1 |
| 54 | Sputum. | " " | 5.0 |
| 70 | Throat. | " " | 5.1 |
| 115 | " | " " | 5.1 |
| 134 | " | " " | 5.1 |
| 140 | " | " " | 5.1 |

TABLE VI—*Continued.*

| Strain No. | Source. | Diagnosis | pH |
|---|---|---|---|
| 9 | | Lobar pneumonia. | 5.1 |
| 11 | Sputum. | " " (Type I). | 5.1 |
| 15 | Throat. | " " | 5.1 |
| 16 | Sputum. | " " | 5.2 |
| 27 | Autopsy (lung). | " " | 5.1 |
| 44 | Throat. | " " | 5.1 |
| 56 | Autopsy (lung). | " " | 5.1 |
| 61 | Sputum. | " " (Type IV). | 5.2 |
| 62 | Throat. | " " | 5.1 |
| 65 | Sputum. | " " | 5.1 |
| 69 | Autopsy (lung). | " " | 5.0 |
| 75 | | " " | 5.1 |
| 87 | Sputum. | " " | 5.2 |
| 95 | Throat. | " " | 5.1 |
| 99 | | " " | 5.1 |
| 120 | Throat. | " " | 5.1 |
| 125 | " | " " | 5.1 |
| 133 | | " " | 5.1 |
| 144 | Throat. | " " | 5.1 |
| 8 | " | Pneumonia. | 5.1 |
| 281 | Sputum. | " | 5.1 |
| 286 | Pleural fluid. | " | 5.1 |
| 6 | Throat. | Incipient tuberculosis. | 5.1 |
| 34 | " | " " | 5.1 |
| 41 | " | " " | 5.1 |
| 46 | " | " " | 5.1 |
| 121 | " | " " | 5.2 |
| 122 | " | " " | 5.1 |
| 129 | " | " " | 5.1 |
| 40 | Pericardial fluid. | | 5.0 |
| 267 | Foot. | Cellulitis. | 5.0 |
| 271 | Blood. | Septicemia. | 5.0 |
| 276 | Pus. | Pelvic abscess. | 5.1 |
| 277 | " (abdomen). | | 5.1 |
| 264 | Blood. | Osteomyelitis. | 5.2 |
| 266 | " | Meningitis. | 5.0 |
| 306 | Spinal fluid. | " | 5.0 |

TABLE VI—*Concluded.*

| Strain No. | Source. | Diagnosis. | pH |
|---|---|---|---|
| SH | 1913 Boston epidemic. | Peritonitis. | 5.0 |
| S8 | 1917 " " | Empyema. | 5.2 |
| 38 | 1912 Baltimore " | Adenitis. | 5.0 |
| C64B | Throat. | | 5.1 |
| C63B | " | | 5.1 |
| 57* | " | Normal. | 4.8 |
| 91 | " | Measles. | 4.8 |
| 103 | " | " | 4.9 |
| 105 | " | Influenza. | 4.9 |
| 288 | Sputum. | Pneumonia. | 4.8 |
| X35B | | Erysipelas. | 4.9 |
| A103B | Throat. | | 4.8 |
| 30 | 1912 Chicago epidemic. | Peritonitis. | 4.9 |

* The eight human strains with a pH of 4.9 and 4.8 have been grouped together without reference to diagnosis.

TABLE VII.

*Final Hydrogen Ion Concentration of Streptococcus hæmolyticus from Cheese.*

| Strain No. | pH | Strain No. | pH |
|---|---|---|---|
| Ch. 1 | 4.3 | Ch. 11 | 4.4 |
| " 2 | 4.4 | " 12 | 4.5 |
| " 3 | 4.3 | " 13 | 4.4 |
| " 4 | 4.4 | " 14 | 4.4 |
| " 5 | 4.4 | " 15 | 4.5 |
| " 6 | 4.3 | " 16 | 4.3 |
| " 7 | 4.5 | " 17 | 4.5 |
| " 8 | 4.3 | " 18 | 4.3 |
| " 9 | 4.5 | " 19 | 4.5 |
| " 10 | 4.5 | | |

*Survey of Streptococcus hæmolyticus.*

With the assurance given that the media and conditions of growth were suitable for a survey of streptococci from various sources, we have completed the examination of 124 strains of *Streptococcus hæmolyticus* of known human origin and 45 from bovine sources, including 19 cultures of hemolytic streptococci isolated from cheese and 26 from milk. These results have been assembled in Tables VI to VIII, and are represented graphically in Text-fig. 2.

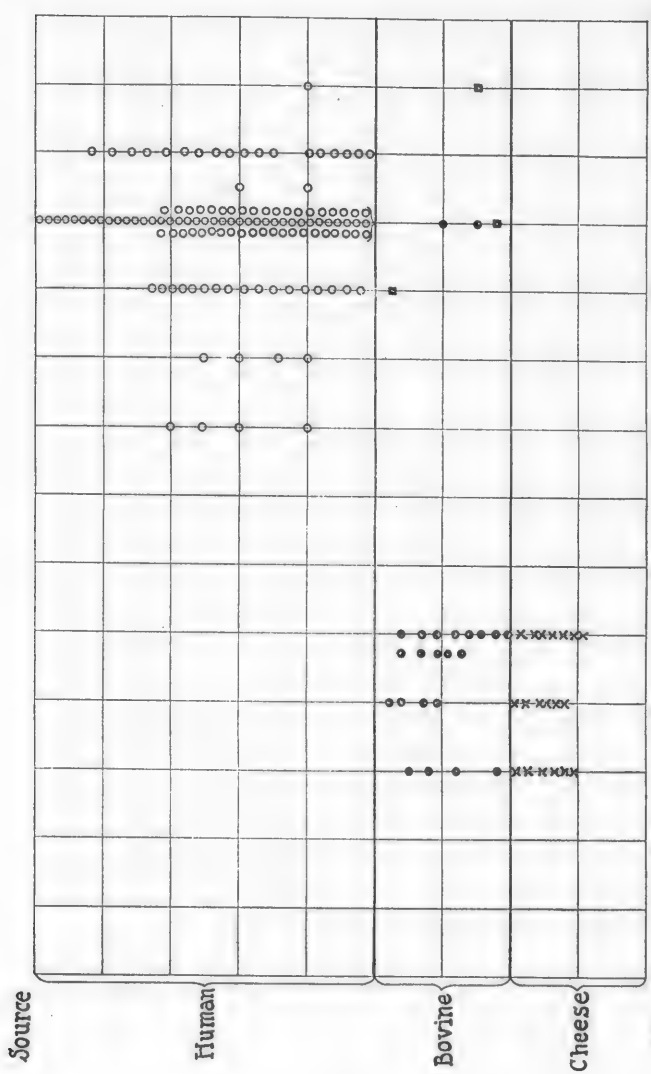
TABLE VIII.

*Final Hydrogen Ion Concentration of Streptococcus hæmolyticus from Bovine Sources.*

| Strain No. | Source. | pH | Strain No. | Source. | pH |
|---|---|---|---|---|---|
| C67 | Mastitis. | 4.5 | M2 | Milk. | 4.3 |
| C57 | " | 4.5 | M41 | " | 4.5 |
| C53 | " | 4.5 | M43 | " | 4.5 |
| C69 | " | 4.5 | V1 | Udder. | 4.4 |
| C59 | " | 4.3 | V2 | " | 4.3 |
| M26 | " | 4.5 | V3* | " | 5.0 |
| M93 | Milk. | 4.5 | V4 | " | 4.3 |
| M53* | " | 5.3 | V5 | " | 4.5 |
| M3 | " | 4.5 | V6 | Milk. | 4.5 |
| M86* | " | 5.1 | V7 | Udder. | 4.4 |
| MJ1* | " | 5.1 | V8 | " | 4.5 |
| M65 | " | 4.5 | V9 | " | 4.5 |
| M1 | " | 4.4 | V10* | " | 5.1 |

* Strains V3 and V10 were isolated by Smith and Brown (5) from the udders of cows during Boston epidemics of septic sore throat and identified by them as human types. Strains MJ1, M86, and M53 were recovered by Jones from milk of cows showing no evidence of mastitis, and were described by him as either human strains which had contaminated the milk or streptococci from the skin or feces of cows. None of these cultures has been tested for pathogenicity. He found that none of the three strains agglutinated in the immune serum prepared by immunization of animals with *Streptococcus hæmolyticus* from bovine mastitis (12).

The results from this study are striking. None of the cheese strains shows a final hydrogen ion concentration less than a pH of 4.5. Of the 26 cultures from bovine sources, 21 had a final reaction between 4.3 and 4.5. Five showed a reaction of from 5.0 to 5.3; of these 5, 2 were received from Dr. J. H. Brown, and were designated by him, from their cultural characteristics, as human streptococci. The other 3 are of questionable diagnosis (foot-note, Table VIII). Of the 124 strains from known human origin, only 8 showed a pH less than 5.0 and none less than 4.8. It must be emphasized that the final hydrogen ion concentration of none of the large number of organisms examined occurred within the zone of 4.5 to 4.8, and only 8 in the zone of 4.8 to 5.0. This is important, for the zone below a pH of 4.5 showed a distinct red color with methyl red, while a reaction above 5.0 showed only a very faint salmon pink.




TEXT-FIG. 2. Difference in final hydrogen ion concentration of human and bovine types of *Streptococcus haemolyticus*.

*Practical Application of the Method of Hydrogen Ion Concentration in the Differentiation of Human and Bovine Types of Streptococcus hæmolyticus.*

The difference in the final hydrogen ion concentration of these two types of organisms has been expressed in terms of the pH values throughout the present discussion. In the practical application of this method, however, it is not necessary to determine the actual final hydrogen ion concentration. Methyl red reacts in such a way at the different final hydrogen ion concentrations reached by human and bovine strains, that no difficulty is experienced in judging these color values directly. At a pH value of 5.0 to 5.2, representing the range of the final hydrogen ion concentration of human strains, the color of this indicator is a faint salmon pink, whereas at a pH of 4.3 to 4.5, the final value for organisms of bovine origin, the color is a decided red. This color difference is so marked that comparison with standard solutions is not necessary and slight experience suffices to permit the determination of the type from the color produced by the direct addition of methyl red to the culture.

In determining the type *Streptococcus hæmolyticus* the following procedure has been adopted. The strain to be tested is grown in test-tubes (7 by $\frac{7}{8}$ inches) containing 5 cc. of 1 per cent dextrose broth. After the maximum growth has been reached, generally within 24 to 48 hours depending upon the size of the inoculum and the suitability of the medium, the culture fluid is diluted with 10 cc. of distilled water, and one drop of 0.1 per cent alcoholic solution of methyl red added. The difference in color, representing the pH values, distinctive for the human and bovine types of *Streptococcus hæmolyticus* is immediately apparent. This reaction is sufficiently constant to be relied upon, and although an occasional atypical strain may be encountered, it constitutes a presumptive test of real value in differential diagnosis.

## DISCUSSION.

The bacteriological methods used at present in the differentiation of human and bovine types of *Streptococcus hæmolyticus*, namely the

determination of their hemolytic and fermentative activity, and pathogenicity for rabbits, are time-consuming and inadequate. In addition, the determination of the titratable acidity produced by these organisms in the fermentation of certain test substances is admittedly unreliable. The corelation of these several reactions, however, together with minor morphological and cultural characters indicates clearly that distinctive differences of a biologic nature exist between hemolytic streptococci found in pathologic lesions of man and those occurring in bovine mastitis, in milk and dairy products. Any method, therefore, for the rapid differentiation of these closely allied organisms would be of inestimable advantage in epidemiological investigation.

From the data recorded in these experiments it appears that the final hydrogen ion concentration of human and bovine hemolytic streptococci is a constant and distinctive characteristic of these organisms. The human type of *Streptococcus hæmolyticus* reaches a final hydrogen ion concentration of pH 5.2 to 5.0, and the bovine type of pH 4.5 to 4.3. It has been found that in the medium described, the concentration of dextrose, 0.5 to 1.5 per cent, does not affect the final hydrogen ion concentration. Acid production in dextrose medium proceeds rapidly and the maximum acidity is reached in 24 to 48 hours, depending on the size of the inoculum and suitability of the medium for growth. This final reaction inhibits growth and does not change on further incubation of the culture. It also appears from these experiments that the final hydrogen ion concentration of *Streptococcus hæmolyticus* is not related to virulence, since no variation was found in the reaction of cultures as originally isolated and of the same cultures after repeated animal passage.

All the cultures studied were of known origin, and on the basis of clinical evidence and bacteriological methods had been previously identified as belonging to one or the other of these two types. By the method described 95 per cent of all the strains examined were accurately and rapidly classified by the determination of their final hydrogen ion concentration in 1 per cent dextrose broth.

The final hydrogen ion concentration of the strains from bovine sources has in no instance fallen in the intermediary zone between the limits distinctive for human and bovine types. Of the five strains of bovine origin giving the same final hydrogen ion concentration

as human strains, two had previously been identified by other methods as belonging to the human type. The other three may be either human strains which have contaminated the milk or streptococci from the skin or feces of cows (foot-note, Table VIII). Further investigation should be made on the final hydrogen ion concentration of hemolytic streptococci from feces and skin of cows.

In the practical application of these facts use has been made of the difference in color of methyl red at the final hydrogen ion concentration of these two types. This change in color is sufficiently distinctive in itself to serve as a direct test for type determination and obviates the necessity of comparative readings with accurately standardized solutions of the phosphates. Because of the accuracy and constancy of the final hydrogen ion concentration, this method offers a presumptive test of distinct value in the classification of hemolytic streptococci.

## SUMMARY.

1. Under the conditions of these experiments, there appears to be a distinct and constant difference in the final hydrogen ion concentration of *Streptococcus hæmolyticus* from human and bovine sources.

2. Of 124 strains of *Streptococcus hæmolyticus* from known human origin, 116 reached a final hydrogen ion concentration of from pH 5.0 to 5.3. Only 8 reached a pH more acid than 5.0 and none more acid than pH 4.8.

3. Of 45 strains of *Streptococcus hæmolyticus* from bovine sources, including 26 strains isolated from milk and the udder of cows, and 19 from cream cheese, 40 reached a final hydrogen ion concentration of pH 4.3 to 4.5. Of the remaining 5 which reached a pH of 5.0 to 5.2, two were of known human type and three of uncertain diagnosis.

4. A rapid and practical application of this method is proposed as a presumptive test in the differentiation of human and bovine types of *Streptococcus hæmolyticus*.

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